CORRELATION OF THE IMMUNOHISTOCHEMICAL EXPRESSION OF HER 2 WITH TUMOUR MORPHOLOGY AND TNM STAGE IN MUCOSAL BIOPSIES AND CORRESPONDING TOTAL OR SUBTOTAL GASTRECTOMY SPECIMENS OF PATIENTS WITH ADENOCARCINOMA

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REGULATION FOR THE AWARD OF THE DEGREE OF MD PATHOLOGY

(BRANCH III)

THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY

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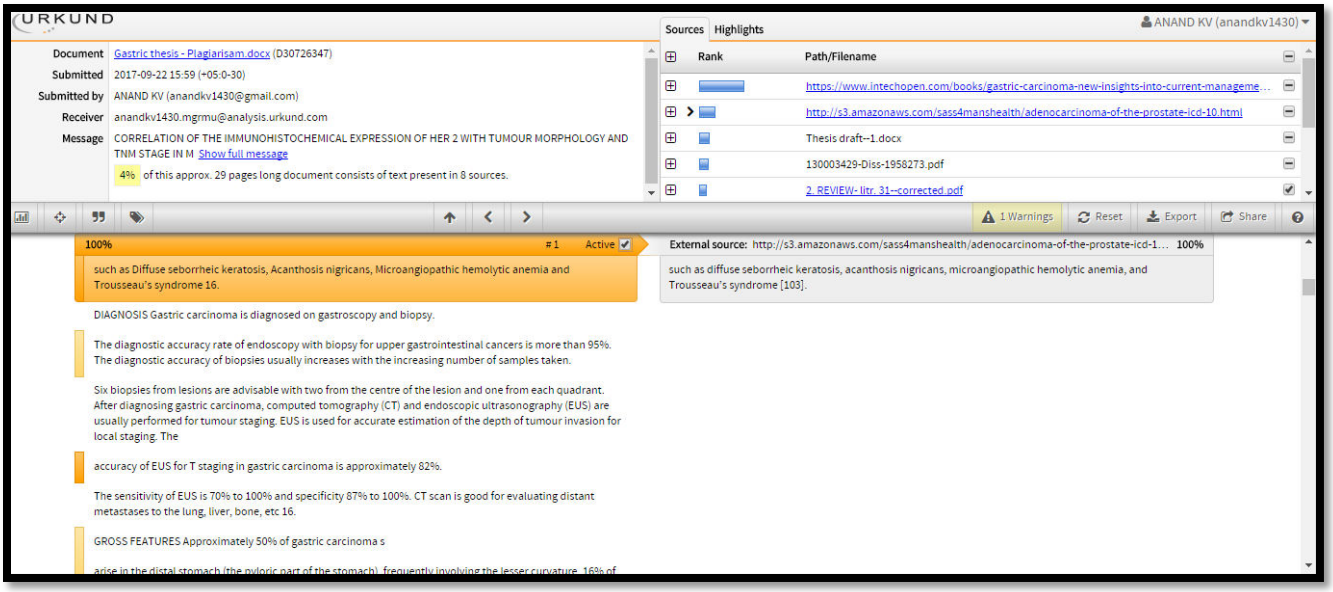
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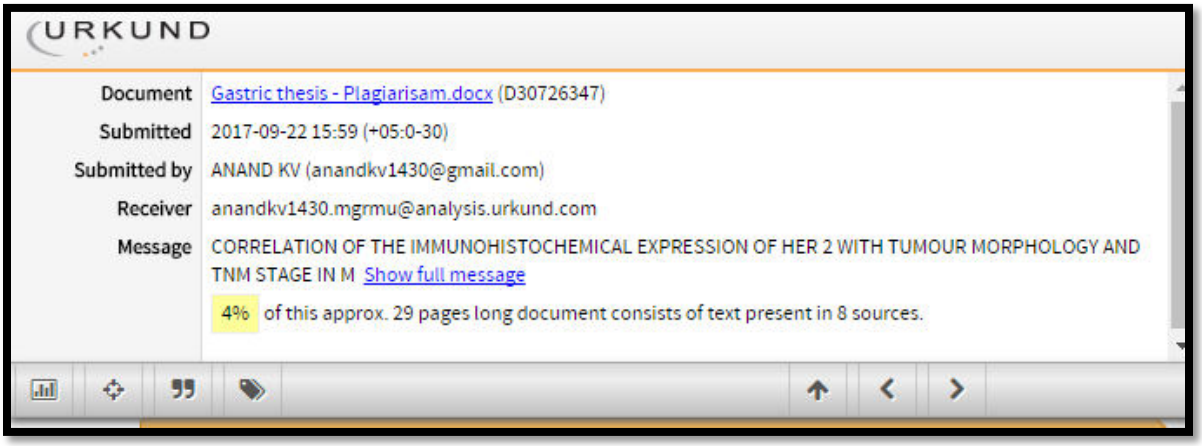
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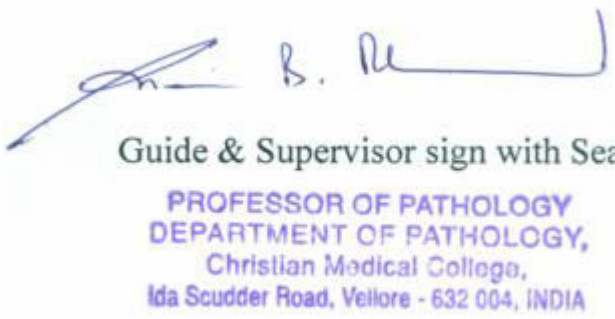

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ABSTRACT

Background &Objectives: Gastric cancer is one of the leading causes of death and the fourth most prevalent cancer in the world. Most cases of gastric carcinoma are detected in advanced stages and are associated with high mortality and bad prognosis. For such advanced diseases, treatment options are limited. Trastuzumab has been approved for metastatic or locally advanced carcinomas arising in the stomach or the gastro esophageal junction in patients with HER2-positive tumours. The multicentre TOGA trial proved that targeted therapy could prolong patient lives by 2.7 months when compared to the standard treatment. There is limited data on the prevalence and behavior of HER2-positive cases among Indian patients. The current study aims to,

1. Compare HER2 expression between matched diagnostic biopsies and surgical specimens of patients with gastric adenocarcinoma in India.

2. Correlate HER 2 expression with important prognostic pathological parameters and to determine the effect of non-Trastuzumab containing neo adjuvant chemotherapy (NAC) on this expression.

3. To study tumour heterogeneity among the HER2 positive Gastric adenocarcinomas

Type of study: prospective observational study.

Materials and Methods: Matched primary gastrectomy sections with corresponding diagnostic biopsies of 72 patients reported from June 1, 2016, to July 30, 2017 were used

from the archives of the Department of Pathology. The immunohistochemical study was conducted using MILD CC 1 protocol perform with anti-HER 2/neu (4B5) Rabbit Monoclonal Primary Antibody, and the Ventana Pathway using automated slide stainer Ventana Bench mark XT. We stained whole-tissue sections with their matching diagnostic mucosal biopsies on the same slide. We comparedHER2expression status with all pathological parameters to assess statistically significant associations by the Chi-square test. HER-2 overexpression (HER2+) was defined by a score 3+ on IHC according to the standardized and validated scoring system of Hoffmann et al. used in most international trials including the TOGA trial.

Results: Paired HER2 status was determined for 72 patients (100%). HER2+ rates were 8.33% on biopsy (6/72) and 9.72% on resection (7/72). The overall HER2 positivity rate was 11.11% (8/72). There was an association between HER2 expression and WHO mixed adeno carcinoma histological subtype (P = 0.009) and presence of lymphovascular invasion (P = 0.045). No association was found between HER2 status and all other pathological parameters. When we independently analyzed the cases, 14/ 58 cases were NAC treated cases, and 3 cases showed HER 2 positivity. In non-NAC patients 4/5 (80%) HER 2 positive cases showed concordance between the biopsy and resection. The remaining 1/5 case showed discordance with a positive shift. In the NAC group 3/3 (100%) HER 2 positive cases showed discordance with 2/3showing negative shift and 1/3 with the positive shift. All 3/5 (60%) treated patients showed tumour

heterogeneity and all three were mixed type. The remaining 2/5(40%) showed homogeneous staining pattern and were of the WHO tubular variant

Conclusion: To our best knowledge this is the first study to analyse HER 2 expression in 72 matched biopsies with the corresponding resections in India and this largest study group compared with other similar studies published in India. Differences between biopsy and resection HER2 expression could be explained by intratumoral heterogeneity and by decreased HER2 expression in surgical sections after NAC in responding patients possibly due to a higher chemo sensitivity of HER2-positive clones. Combining the analysis of biopsy and resections could optimize the selection of Trastuzumab-eligible patients in case of advanced gastric adenocarcinoma particularly in previously NAC-responding patients having a mixed histological type of tumour with lymphovascular invasion.

Keywords: Her2, gastric cancer, neoadjuvant chemotherapy, Immunohistochemistry.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

CISH: CHROMOGENIC IN SITU HYBRIDIZATION

ELISA: ENZYME LINKED IMMUNOSORBENT ASSAY

FISH: FLUORESCENCE IN SITU HYBRIDIZATION

GEJ: GASTROESOPHAGEAL JUNCTION

H.PYLORI: HELICOBACTER PYLORI

HER2: HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2

IHC: IMMUNOHISTOCHEMISTRY.

NACT: NEOADJUVENT CHEMOTHERAPY

TOGA: TRASTUZUMAB FOR GASTRIC CANCER TRIAL

WHO: WORLD HEALTH ORGANISATION.

INTRODUCTION

Gastric carcinoma is one of the most common tumours that cause death globally and most cases are diagnosed only in very late stages are associates with a high incidence of tumour metastasis. For such cases, the scope of surgical resection is restricted. Trastuzumab is a monoclonal antibody used in the management of tumours which interferes with HER 2 receptor function and increases overall survival. Our current study correlated the immunohistochemical (IHC) expression of HER 2 with variables such as tumour classifications, tumour topography, tumour grading and TNM stage in matched mucosal biopsies and gastric resection specimens of the patients with adenocarcinoma. The primary objective of our study was to analyse the prevalence of HER 2 expression in gastric carcinoma in our hospital and nature of tumorogenesis including tumour heterogeneity. Cases were recruited from 1 June 2016 to 30 July 2017.

AIM & OBJECTIVES

- Study the HER 2 over expression by immunohistochemistry in gastric adenocarcinoma patients diagnosed in our hospital.

- Correlate the expression of HER 2 by immunohistochemistry in gastric carcinoma with pathological parameters

- Correlate the expression of HER 2 in mucosal biopsies and surgical resections of the same patients.

REVIEW OF LITERATURE

EPIDEMIOLOGY

GLOBOCON 2012 statistical data revealed about 951,600 newly diagnosed gastric cancer cases. In 2012 deaths due to gastric carcinoma worldwide was around 720,000 [1]. Gastric carcinoma is the fifth commonest malignancy in the world and the third most frequent malignancy causing death. The sex ratio of gastric malignancy is 2:1 in males and females. The Eastern part of Asia (Korea> Mongolia>Japan> China) and Europe have the highest incidence of gastric carcinoma. The lowest incidence has been recorded in Africa and Northern America [1]. Since 1990 interestingly, there is a reduction in gastric carcinoma mortality rates and the global incidence has been reducing gradually from the late eighties [2](See Table 1). Diffuse type gastric carcinoma is relatively increasing however, compared to intestinal type[3]. A factor directly linked to this steady global decline of gastric carcinoma could be the increased availability of fresh vegetables, fruits and the availability of refrigerators which increase freshness and reduce salt based preservation. The invention of antibiotics and the improved quality and awareness of sanitation are other main factors which reduce chronic H. pylori infection. Reduction in smoking also reduced the prevalence in developed countries[4]. Gastric carcinoma ranks high among male cancers in South India. The prevalence of H. pylori infection is alarmingly high in India, and could be the reason the burden of stomach cancer is very

high. In addition to H.pylori, almost all of etiological risk factors for gastric carcinoma are present among the Indian population[5].

Table 1.Incidence comparison between GLOBOCON 2012, 2008, 2002

GLOBOCON	2012		2008		2002	
	male	Female	male	female	male	Female
East Asia	35.4	13.8	42.4	18.3	62.1	26.1
E. Europe	20.3	8.9	22.2	9.7	29.6	12.8
S.America	14.2	7.0	17.3	8.4	24.2	12.2
West Asia	11.8	7.3	12.6	6.7	11.6	6.4
S. Europe	11.7	5.9	14	6.8	18	8.7
C.America	10.6	8.2	12.7	9.3	15.2	10.8

*Age per standerdised rate per 100,000. E- Easter, S-Southern, C-Central

ANATOMY OF THE STOMACH

The stomach is a part of the gastrointestinal system which is continuous with the duodenum distally and the esophagus proximally. Its functions include food storage and also initiating the process of digestion. The stomach is divided into four parts Figure PYLORUS /ANTRUM: the pyloric antrum leads to the pyloric canal. BODY: The portion between the pyloric antrum and fundus. FUNDUS: the proximal part of the stomach above the Gastro esophageal Junction. CARDIA: The part surrounding the

cardiac orifice. The lesser curvature is concave and shorter than the greater curvature which is convex and much longer. The incisura angularis demarcates the body and pylorus. Because of the short length of the lesser curvature, tumours in this site require total gastrectomy. The stomach wall comprises of four layers namely the mucosa, sub mucosa, muscularis propria and serosa. The anterior peritoneal lining is part of the greater sac, and the peritoneal lining on the posterior wall forms part of the lesser sac. There is minimal peritoneal lining on the posterior aspect of the gastro esophageal junction.

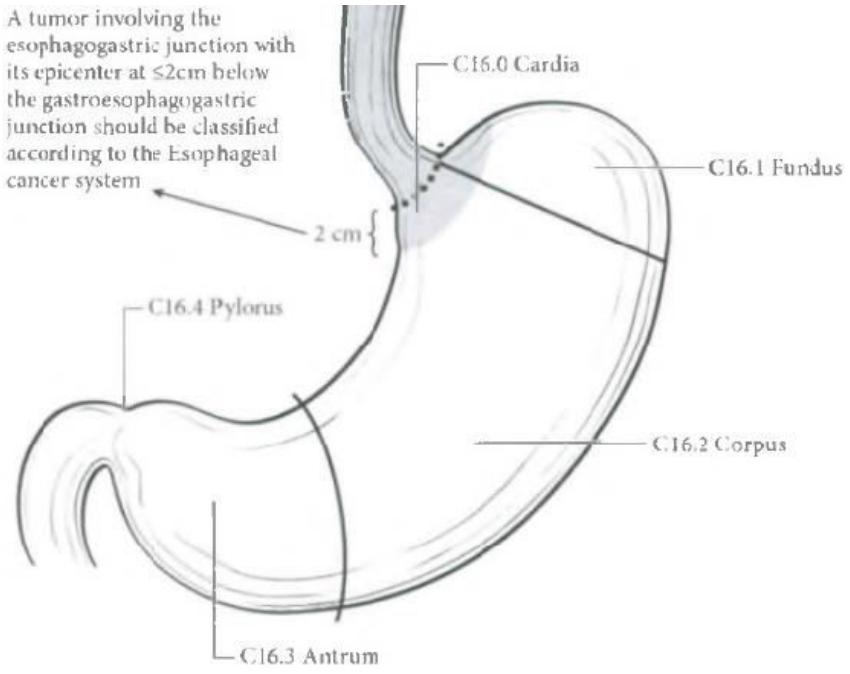

Figure 1.Anatomic sub sites of the stomach

DEFINITION OF ADENOCARCINOMA STOMACH

Adenocarcinoma of the stomach is a "Malignant gland forming neoplasm of the stomach, exclusive of the GEJ".

As per the latest AJCC 8th edition, tumours involving the GEJ with the tumour epicenter no more than 2 cm into the proximal stomach are staged as esophageal cancers. GEJ tumours with their epicenter located greater than 2 cm into the proximal stomach are staged as stomach cancers. Cardia cancers not involving the GEJ are considered as stomach cancers[6].

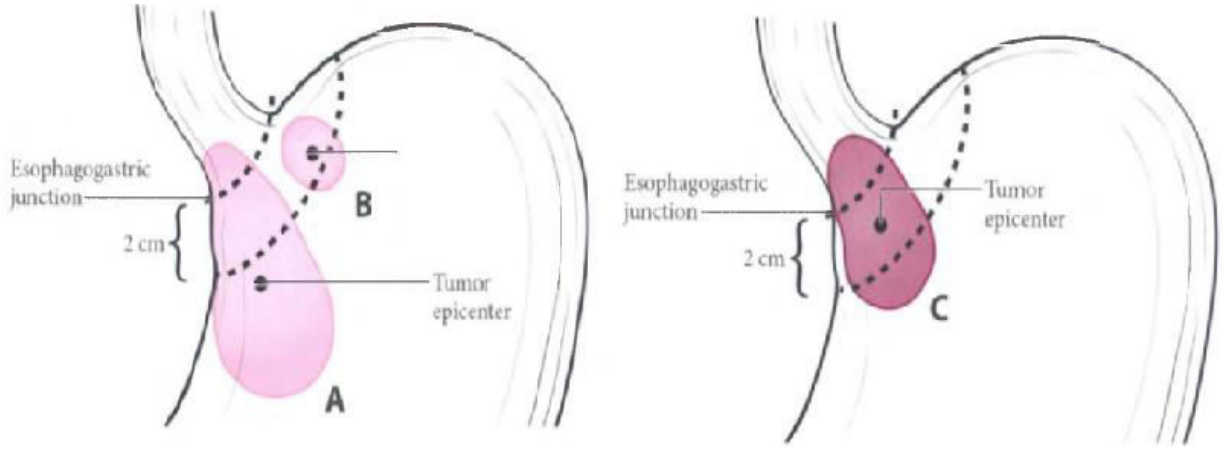

Figure 2.Definition of Gastric and GEJ carcinoma. (A) EGJ tumors with their epicenter located >2 cm into the proximal stomach are staged as stomach cancers. (B) Cardia cancers not involving the EGJ are staged as stomach cancers. (C) Tumors involving the EGJ with their epicenter <2 cm into the proximal stomach are staged as esophageal cancers.

ETIOLOGY

Age

Most patients presenting with gastric carcinoma are between50 and 70 years[7]. Early onset gastric carcinoma (<40 years) is rare and has different etiologies. These early onset cases differ in their sex incidence (with either an equal male: female ratio or female predominance), morphology, (diffuse type rather than intestinal type), poor differentiation and have a poor prognosis[8].

Gender

Gastric carcinoma shows a strong male predominance, with an approximately 2:1 male: female ratio[6].The male prevalence of gastric carcinoma is greater in high incidence areas. There is a consistently higher male: female ratio in gastric carcinomas arising in the cardia compared to those affecting the distal stomach (antrum and pylorus). Early gastric carcinoma shows a 1:1 or 0.9:1 male: female ratio[9]. Globally gastric adenocarcinomas are more common in Asians than in whites[6].

AETIOPATHOGENESIS

Gastric carcinoma is a multifactorial and multistep disease that often involves a ladder wise progression starting from normal gastric mucosa to chronic gastritis, atrophic gastritis and intestinal metaplasia. Thereafter multiple host and genetic factors contribute towards the development of dysplasia and carcinoma in situ, and ultimately to invasive

carcinoma[10] (See figure3). Risk factors commonly involved in the evolution of gastric carcinoma are discussed in three broad categories:

Environmental factors

H.pylori infection is the prime culprit involved in the multiple steps leading gastric carcinoma by producing a lot of factors that disrupt the function of normal mucosal barriers and act as cancer promoters. These include the production of urease, Cytotoxin associated gene A (CAG A), Vacuolating gene A (Vac A), Phospholipase production, Protease production, Upregulation of host immunity and hypochlorhydria, increased free radical production, decreased gastric anti oxidant levels, increased epithelial cell proliferation and increased risk of mutations[11]. H.pylori activates the WNT signalling pathway with the help of β catenin. Diets rich in salts and nitrites and diets low in antioxidants also form N nitroso compounds supporting carcinogenesis[12]

Host factors

Intestinal metaplasia and chronic atrophic gastritis are the most common risk factors found in hosts[13]. Partial gastrectomies with bile reflux, the presence of gastric adenomas with high-grade dysplasia, Autoimmune gastritis and Menetriers disease are other precursor lesions of gastric adenocarcinoma[14].

Genetic factors

CDH1 gene (encoding E cadherin) germline mutation in individuals with a family history of gastric adenocarcinoma, the presence of Familial Adenomatous Polyposis and Hereditary Non-polyposis Cancer Syndrome (HNPCC) are primary genetic risk factors associated with the development of this malignancy[15].

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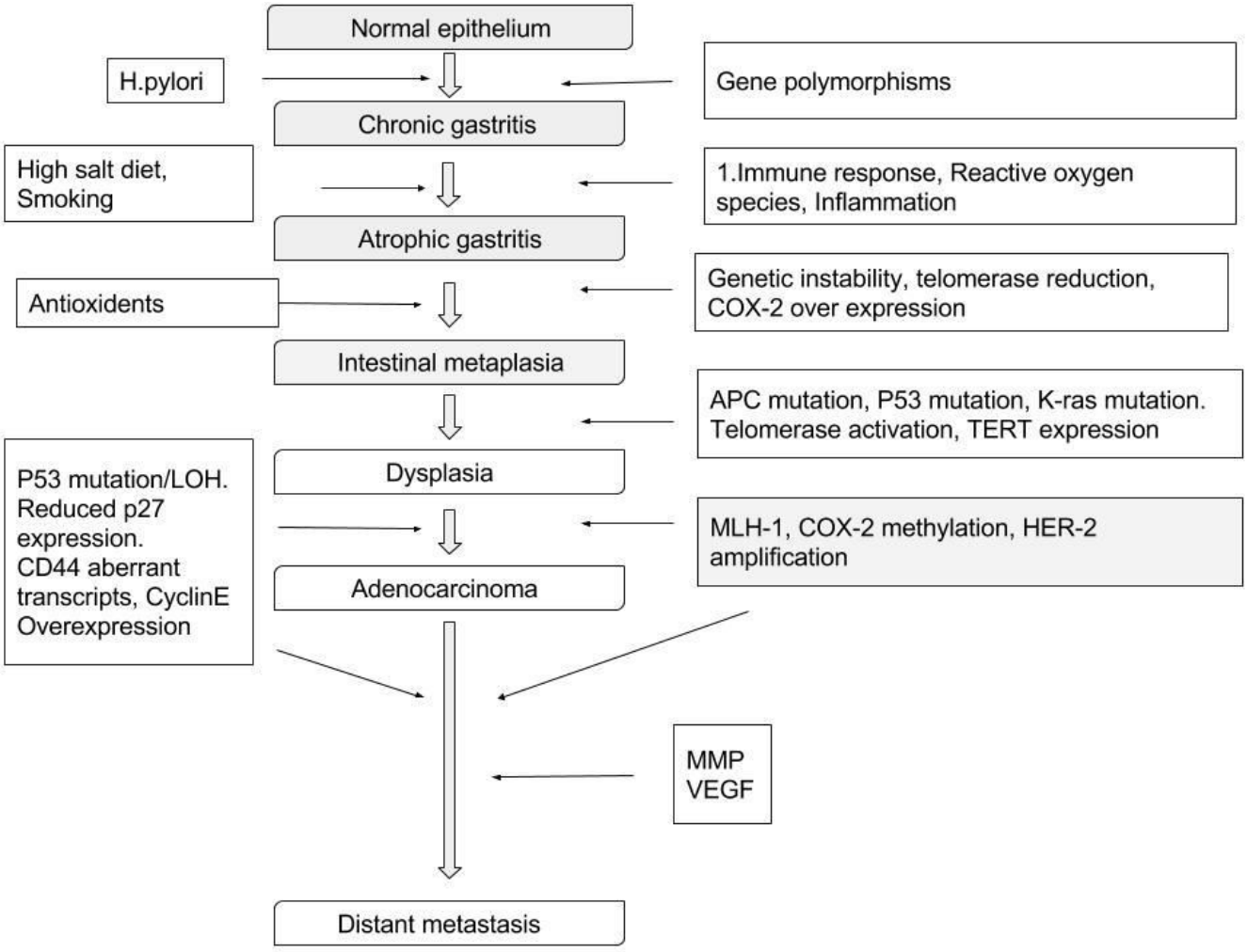

Figure3.Multistep gastric pathogenesis.

.CLINICAL FEATURES

Gastric adenocarcinoma is a disease of insidious onset with variable clinical features and a frequent initial asymptomatic period. The initial presentation is often nonspecific with vague upper gastrointestinal symptoms including anorexia, nausea, vomiting and dyspepsia. Patients with more advanced lesions complain of epigastric mass, dysphagia, loss of weight, haematemesis and melaena. Sister Mary Joseph nodule which is a subcutaneous umbilical nodule may be present and represents a periumbilical metastatic deposit. Additionally, there may be supraclavicular lymphadenopathy, commonly known as Virchow Trossier node, due to lymph node metastasis. Distinctive bilateral ovarian gastric adenocarcinoma metastases are known as Krukenburg tumours. Paraneoplastic syndromes also are seen commonly such as Diffuse seborrheic keratosis, Acanthosis nigricans, Microangiopathic hemolytic anemia and Trousseau's syndrome[16].

DIAGNOSIS

Gastric carcinoma is diagnosed on gastroscopy and biopsy. The diagnostic accuracy rate of endoscopy with biopsy for upper gastrointestinal cancers is more than 95%. The diagnostic accuracy of biopsies usually increases with the increasing number of samples taken. Six biopsies from lesions are advisable with two from the centre of the lesion and one from each quadrant. After diagnosing gastric carcinoma, computed tomography (CT) and endoscopic ultrasonography (EUS) are usually performed for tumour staging. EUS is used for accurate estimation of the depth of tumour invasion for local staging. The

accuracy of EUS for T staging in gastric carcinoma is approximately 82%. The sensitivity of EUS is 70% to 100% and specificity 87% to 100%. CT scan is good for evaluating distant metastases to the lung, liver, bone, etc[16].

GROSS FEATURES

Approximately 50% of gastric carcinoma s arise in the distal stomach (the pyloric part of the stomach), frequently involving the lesser curvature. 16% of gastric carcinoma s occurs in the proximal stomach (cardia, the upper third of the body and fundus)

CLASSIFICATION SYSTEMS

Numerous classifications for gastric adenocarcinoma exist, based on macroscopic and microscopic features. These include The World Health Organization (WHO) system, Lauren classification, Ming classification and the Goseki classification. Staging is the most significant predictor of the gastric carcinoma patient's survival however, rather than of any of the pathological classification systems.

Macroscopic - Bormann classification[17]

The Type 1: Polypoid, Type 2: Fungating, Type 3: Ulcerated and Type 4: Infiltrating:

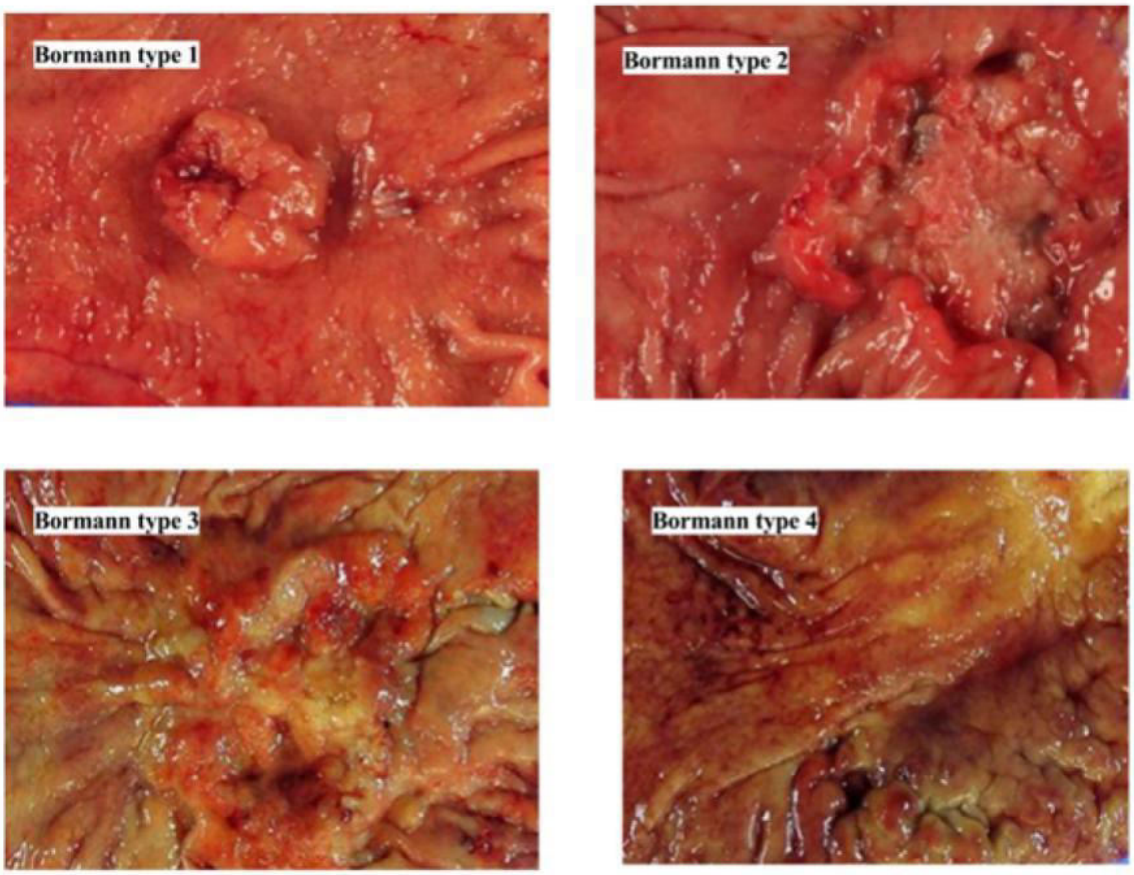

Figure 4.Bormann classification

Microscopic classification

Gastric adenocarcinoma accounts for approximately 95% of all malignant gastric neoplasms. Gastric carcinoma is well known for its heterogeneity and complexity in morphologic characteristics.

In 1965 Lauren described the first primary histopatholgical classification system[18]. This classification divides gastric carcinomas morphologically into two types: Diffuse type gastric carcinoma and intestinal type gastric carcinoma.

The Lauren classification system[18]

The intestinal type of gastric carcinoma is composed of large pleomorphic, mitotically active epithelial cells with large nuclei, prominent nucleoli and variable amounts of intracytoplasmic mucin. The tumour cells form glands, nests, sheets, tubules and may demonstrate papillary architecture. The diffuse type is predominantly composed of poorly cohesive or discohesive epithelial cells with mild nuclear hyperchromasia and minimal pale eosinophilic to clear cytoplasm, often infiltrating into a desmoplastic stroma. Signet ring morphology is often apparent. Gland formation is inconspicuous but appreciated within the superficial regions of a tumour (see Table 2).

Modified Lauren classification system[19]

In this classification both Lauren's pathological classification and the anatomical location of gastric cancer are included, forming three tiers of classification (see Table 3).

Ming Classification[20].

Ming proposed (1977) another classification system of gastric carcinoma based on the expanding vs. infiltrative nature of the tumour growth and invasion pattern. This is an important indicator of biological behavior with the expanding type adenocarcinoma growing predominantly by expansion with a sharply delineated periphery, and resulting in a nodular growth of a tumour, in contrast to the infiltrative type tumours that show diffuse infiltration of tumour cells into the layers of the gastric wall without forming masses or nodules.

Table 2.Difference between intestinal and diffuse type gastric adenocarcinomas

Features	Intestinal	Diffuse
Age	Old age	Young age
Sex	M > F	M = F
Risk factors	Helicobacter pylori infection, high salt diet, and smoking	CDH1 gene mutation
Precursors	Adenoma or dysplasia	Tubule neck dysplasia or signet ring cell carcinoma in situ
Surrounding gastric mucosa	Atrophic gastritis with intestinal metaplasia	Non-atrophic gastritis or nonmetaplastic mucosa
Common location	Antrum and angulus	Corpus and whole stomach
Gross feature	Exophytic lesion	Ulcerative lesion and linitis plastic
Microscopy	Well - developed tubular architecture	Discohesive cells or signet ring cells
Routes of spread	Hematogenous spread	Direct invasion into the surrounding organs

Table 3.Modified Lauren classification system

Modified Lauren	Lauren	Anatomical location
PND	Intestinal type	Bulk (> 80%) located in the gastric fundus/ cardia. These tumours extended up to the GEJ.
D	Diffuse and mixed type	located anywhere in the stomach
DND	Intestinal type	Bulk was usually in the distal stomach, although they could extend up to the mid body of the stomach or down to the pylorus

PND- Proximal non diffuse, D- diffuse, DND- Distal non diffuse

Goseki classification[21]

Classification system of gastric carcinoma based on the degree of tubular differentiation and the amount of intracellular mucin production

Table 4.Goseki classification

Goseki	Tubular differentiation	Intracellular mucin
Group I:	well-differentiated	mucin poor
Group II:	well-differentiated	mucin rich
Group III:	poorly differentiated	mucin poor
Group IV:	poorly differentiated	mucin rich

The WHO(2010) classification system[22].

World Health Organization (WHO) proposed a classification system based on traditional histopathology features and the degree of differentiation of gastric carcinoma.

Papillary adenocarcinoma	Tubular adenocarcinoma
Mucinous adenocarcinoma	Signet ring cell carcinoma
Poorly cohesive carcinoma	Mixed adenocarcinoma
Parietal cell carcinoma	Adenosquamous carcinoma
Carcinoma with lymphoid stroma	Hepatoid adenocarcinoma
Squamous cell carcinoma, NOS	Lymphoepithelial carcinoma
Medullary carcinoma, NOS	Undifferentiated carcinoma

(For the histopathology of individual subtype with ICD definition, see Annexure 8)

TNM STAGING[6].

Depth of invasion

X: Cannot be assessed

pT0: No evidence of a primary tumour

PTis: Carcinoma in situ/high-grade glandular dysplasia

pT1: Tumor invades lamina propria, muscularis mucosae, or sub mucosa

pT1a: Tumor invades lamina propria or muscularis mucosae

pT1b: Tumor invades sub mucosa

pT2: Tumor invades muscularis propria

pT3: Tumor invades subserosal connective tissue, without involvement of visceral peritoneum or adjacent structure

pT4: Tumor invades serosa (visceral peritoneum) or adjacent structures.

pT4a: Tumor invades serosa (visceral peritoneum)

pT4b: Tumor invades adjacent structures (spleen, transverse colon, liver, diaphragm, pancreas, abdominal wall, adrenal gland, kidney, small intestine, and retroperitoneum.)

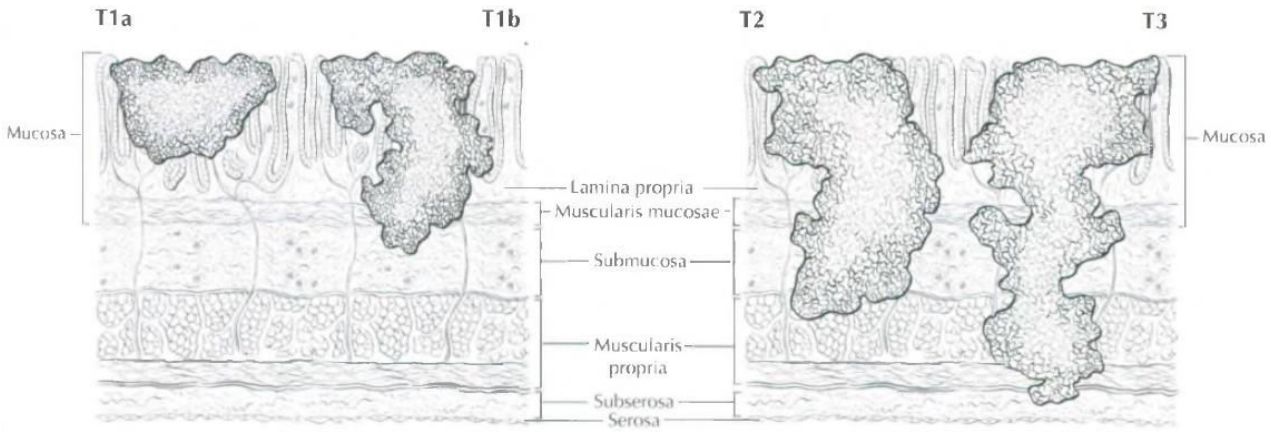

Figure 5.T1 and T2 defintion.T1a is defined as tumor that invades the lamina propria. T 1b is defined as tumor that invades the sub mucosa. T2 is defined as tumor that invades the muscularis propria, whereas T3 is defined as tumor that extends through the muscularis propria into the subserosal tissue[6]

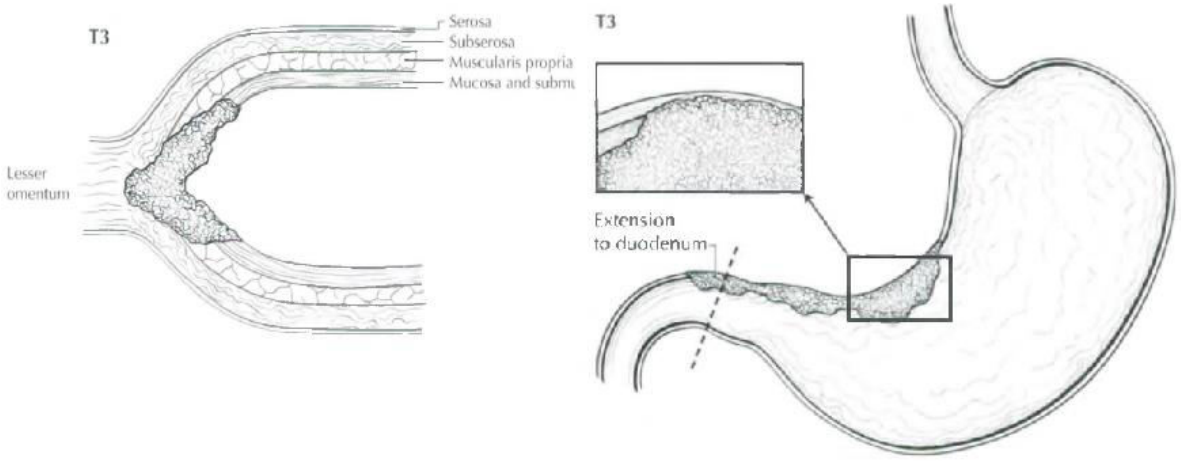

Figure 6.T3 definition. T3 is defined as tumor that invades the subserosal, shown here is invading the lesser omentum without involvement o f the serosa (visceral peritoneum). Distal extension to duodenum does not affect the T3 category[6]

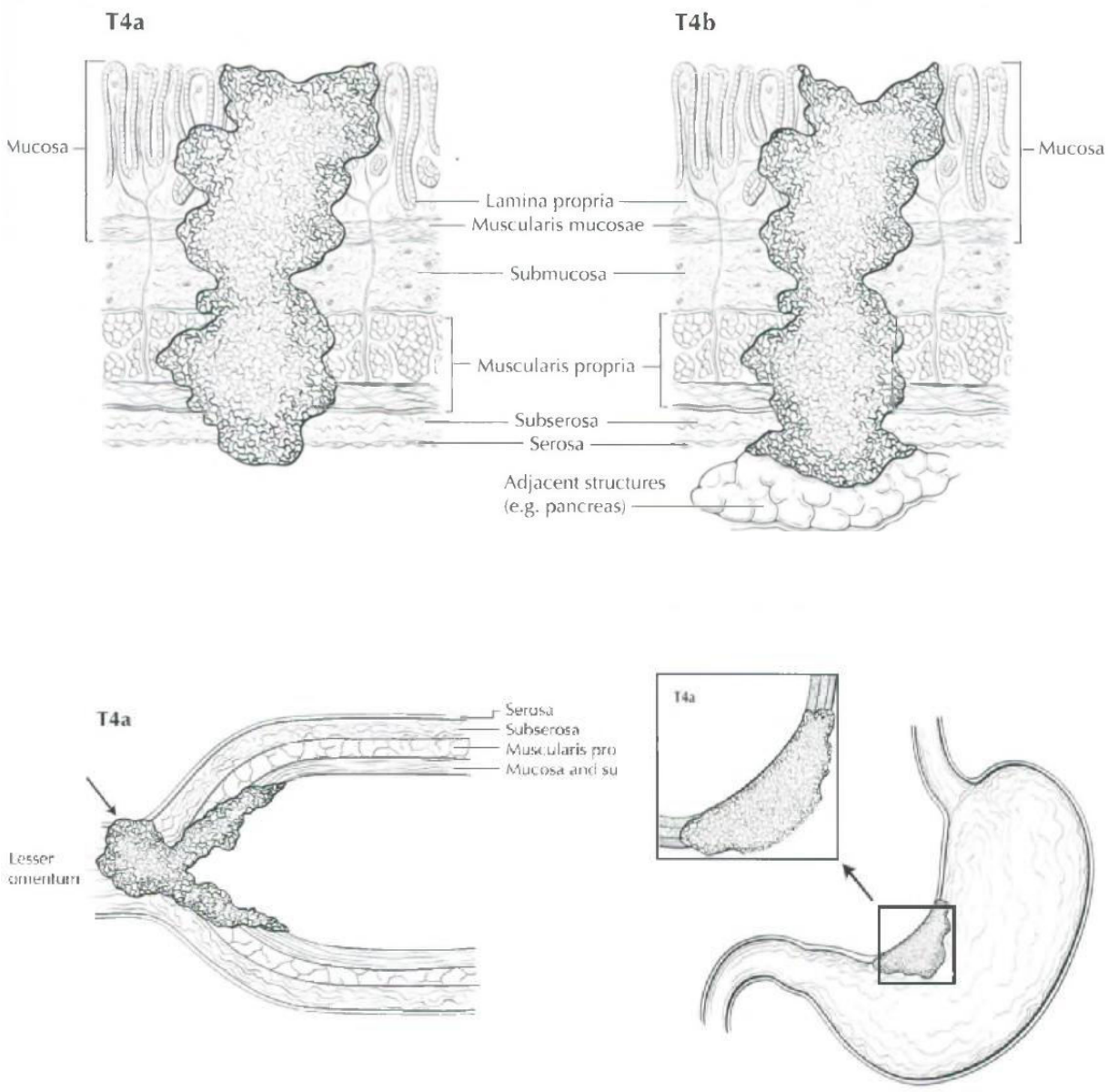

Figure 7.T4a and T4b definition.T4a is defined as tumor that penetrates the serosa (visceral peritoneum) without invasion of adjacent structures, whereas T4b is defined as tumor that radially invades adjacent structures, shown here invading the pancreas[6]

Regional Lymph Nodes (pN)

NX: Cannot be assessed

pN0: No regional lymph node metastasis

pN1: Metastasis in 1 to 2 perigastric lymph nodes

pN2: Metastasis in 3 to 6 perigastric lymph nodes

pN3: Metastasis in 7 or more per gastric lymph nodes

pN3a: Metastasis in 7 to 15 perigastric lymph nodes

pN3b: Metastasis in 16 or more perigastric lymph nodes

Distant metastasis (pM1)

A) Positive peritoneal cytology

B) Nonregional lymph nodes (hepatoduodenal, retro pancreatic, mesenteric, and para-aortic)

C) Peritoneal surfaces (Nodules implanted on the peritoneal surface are considered distant metastases (M1).

D) Adjacent organs (spleen, transverse colon, liver, diaphragm, pancreas, abdominal wall, adrenal gland, kidney, small intestine, and retroperitoneum)

Note: Mx deleted from AJCC 8th edition

TUMOUR GRADING[6]

Grading is based on the 8th edition TNM tumour staging system. According to this system, gastric carcinomas are graded based on the extent of glandular differentiation.

Grade X Cannot be assessed

Grade 1 well differentiated (greater than 95% of a tumour composed of glands)

Grade 2 moderately differentiated (50% to 95% of a tumour composed of glands)

Grade 3 poorly differentiated (49% or less of a tumour composed of glands)

Signet-ring cell carcinomas are high grade and are classified as grade 3.

Small cell neuroendocrine carcinomas and undifferentiated carcinomas are classified as grade 4.

HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2

The HER2 protein also is known by other names like p185 or ErbB-2 is a 185-kDa transmembrane tyrosine kinase receptor. It is part of a family of 4 receptors (ErbB1-4), which have an effect on cancer pathogenesis[23]. In 1985, HER 2 was found to be amplified in breast carcinoma[24]. HER2 was the first molecule to be the focus of targeted therapy in a solid tumor. HER2 targeted molecule Trastuzumab, was approved by FDA for metastatic breast cancer in 1998, and for adjuvant chemotherapy for breast cancer in 2006[25]. HER 2 protein is encoded by the HER proto-oncogene located on the long arm

of chromosome 17[26]. All four members of the ErbB family play a role in cancer progression by forming homo-dimers with another similar molecule or a heterodimer with other members of the ErbB family. Except HER 2, all other dimerizations are ligand induced. However, HER 2 can dimerize without a ligand and hence it is the preferred dimerizing partner for other members of the ErbB family. HER 2 receptor plays an important role because this receptor is constitutively active as a result of its special conformation. When the HER 2receptoris over expressed, it will be the preferred binding partner for other family members and triggers signal transduction which affects cell growth, apoptosis, metastasis and angiogenesis in breast cancer. Studies suggest that the HER2 receptor plays a similar role in gastric cancer[27]. Due to this unique property, it is a primary driver in tumour proliferation and cancer cell survival[28]. Therefore HER 2 is a prime target in appropriately selected patients. HER 2 overexpression in gastric carcinoma was first described in 1986[24]. In literature, the overexpression of HER 2 has been reported worldwide in the range of 7% to 44 %[29]. HER 2 expression is associated with a worse prognosis [30]. A landmark trial using Trastuzumab as a therapeutic option in gastric carcinoma was the ToGA study. The results of this study showed a significant positive survival in patients with gastric carcinoma. The study administered Trastuzumab along with chemotherapy and compared the result with chemotherapy alone [31]. The results of this study proved that adjuvant therapy with targeted Trastuzumab is useful in metastatic gastric carcinoma. After that studies focused on assessing the role of Trastuzumab in non-metastatic gastric carcinoma. Phase II trials give very good results in the management of advanced gastric carcinoma with Trastuzumab [32]. There is a high

chance that Trastuzumab along with other targeted molecules will soon become an important part of the treatment of non-metastatic gastric carcinoma [33]

HER 2 AND GASTRIC CANCER

Different studies have found HER 2 overexpression in gastric and gastro esophageal adenocarcinomas with increasing depth of invasion, lymph node involvement, distant metastases and poor survival. However, there is conflicting information and not all studies have shown a clear association between HER2 over expression and poor prognosis. HER2 protein overexpression and gene amplification are much more heterogeneous in gastric cancer compared to breast cancer. Prevalence of HER 2 show wide variation within and between populations. HER 2 over expression is usually more prevalent in the intestinal variant than diffuse variant[29]. In the Indian population, the expression of HER 2 has been reported to vary from 21 to 44%[30].

HER 2 STATUS ASSESSMENT

Accurate assessment of HER 2 status is essential to make sure that selected patients benefit from targeted therapies. HER 2 status is typically measured by immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH). IHC is more frequently utilized for HER 2 assessment due to its wider availability and lower cost. Hoffman et al developed one standardized validated scoring system for HER 2 assessment in gastric cancer[31]. Cells that stain with a score of 0 or +1 are considered negative. A score of +3 is confirmed over expression, while +2 denote an equivocal positive score. Hoffman et al. have proposed grading criteria for the interpretation of HER2 staining by IHC in gastric cancer, which is different from the criteria, used for breast cancer staining. There were differences in the interpretation of biopsy and resection specimens also. Based on the staining pattern, four scores namely 0, 1+, 2+ and 3+ are given.0 and 1+ staining is considered negative for HER 2.3+ staining as per the given criteria are considered positive for HER2.2+ staining is classified as equivocal and requires further testing using alternate methods like fluorescence in-situ hybridization (FISH), silver in-situ hybridization (SISH) or chromomeric in-situ hybridization (CISH). Particulars of the HER 2 IHC staining is as follows: Primary antibody: Ventana (Pathway) anti HER 2/neu (4B5) Rabbit Monoclonal Primary Antibody.

Procedure -MILD- 32 CC1 PROTOCOLIHC Stainer- Automated Ventana Benchmark XT (Standard operating protocol in Annexure 6).

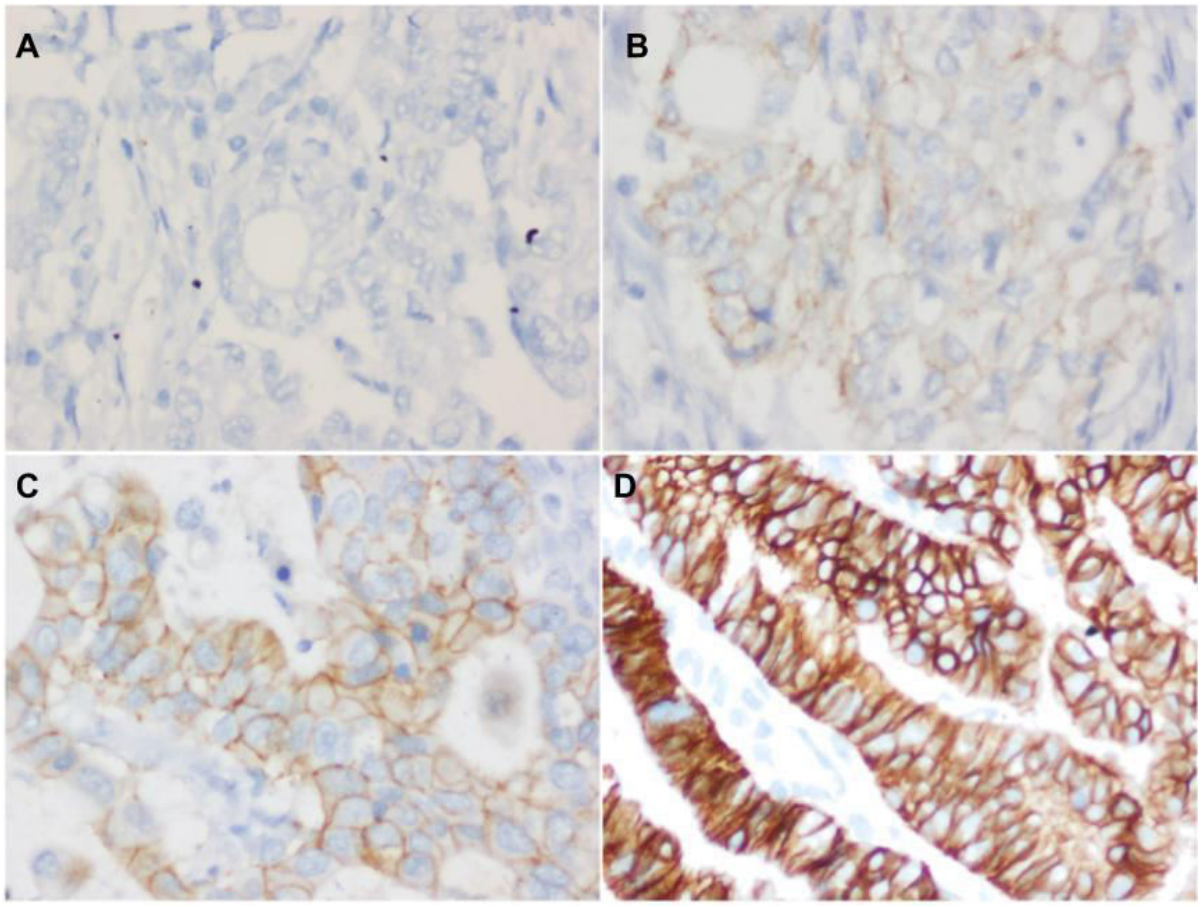

Figure 8.HER 2 IHC scoring system. A. 40x: HER2 0, No staining of tumor cells. B.40x: HER2 1+, Weak incomplete staining of >10 % cells, C.40x: HER2 2+, Weak complete staining of >10 % cells. D. 40x: HER2 3+ Strong complete staining of >10 % cells[31]

Table 5.Hoffman etal scoring for HER2 in gastric cancer

Staining	Surgical specimen	Biopsy	Interpretation
0	No reactivity or membranous reactivity in <10% of tumour cells	No reactivity or no membranous reactivity in any tumour cell	Negative
1+	Faint or barely perceptible membranous reactivity in ≥10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster with a faint or barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Equivocal
3+	Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Positive

Hoffmann etal[31]

HER 2 IHC TESTING AND VARIABLES.

Variability in HER2 testing can arise from pre-analytic, analytic, and post-analytic factors. The relevance of these factors varies according to the measurement technique (e.g., IHC vs. FISH) but each may affect the accuracy, reliability, and reproducibility of results.

Pre-analytic factors

The time of fixation is one major factor. Biopsies or resection specimens must be placed in the recommended fixative as quickly as possible to retain proper antigenic properties. The minimum fixation time recommended is 6–8 hrs for a small biopsy and for surgical resections, 24hrs to 48 hrs. Prolonged fixation also should be avoided (>48 hrs). Prolonged fixation will reduce the staining pattern of HER 2 and leads to false negative results or equivocal results[32]. Mucosal biopsies often receive insufficient fixation, which results in false negative results. The ASCO/CAP Guidelines recommend recording information related to these pre analytic factors as standard procedure[33].

Analytic factors.

Formalin adversely affects epitopes, and proper antigen retrieval steps are required for antibodies detection[32]. Tissue staining patterns will be affected by the antigen retrieval solution and its composition. Automated immunostaining methods are always superior because they reduce all variables associated with technical factors. Even with

Automation however, there are potential sources of error including differences in the optical density of blocks[32].

Post-analytic factors.

Post-analytic factors relate to the interpretation of assay findings, image analysis, reporting, and ongoing quality assurance. Interpretation and cut off values are a primary source of variability both within and between laboratories. Assessing parameters for IHC scoring is greatly influenced by inter- observer and intra-observer variability and are also affected byte use of positive controls for different levels of staining with each batch[34]. The 0–3+ scoring system used to assess HER2 immunostaining differs from other scoring systems used to define cutoffs with other IHC markers. Interpretation is usually performed manually and results can vary depending on the experience and alertness of the observer. Scoring with FISH and newer HER2 Testing techniques are more objective and quantitative than with IHC. Image analysis has been proposed as a means of improving the objectivity of IHC interpretation and reducing intra-observer variability[34].Genetic factors complicating HER2 test interpretation are discussed under HER 2 and heterogeneity.

HER 2 AND HETEROGENEITY

Heterogeneous gene amplification results from two or more distinct or fusion clones of tumour cells displaying different gene amplification patterns. There will be mixed areas with amplified and absence of amplified HER2. This variation in amplification can

results in discordance in IHC or FISH assessment, directly affecting the HER2-targeted therapy[35]. Gastric carcinoma is well known for heterogeneity in HER2 expression which is detected mainly in IHC 2+ cases or mixed histological types[36]. Overexpression of HER2 protein is associated aggressive biological behavior[37]. From sampling to scoring, heterogeneity will adversely affect test results, including the incidence of false negative status. More than intratumoral heterogeneity, heterogeneity of HER2 status between primary and metastatic gastric tumours is common [38]. This difference suggests that HER2 amplification and overexpression can occur de novo in distant metastases in late stage disease and that genetic divergence occurs at the time when in situ cancers progress to invasive cancers. This could also impact patients' eligibility for HER2-targeted therapy[39]. Therefore, in stage IV disease, HER2 testing should ideally be performed on samples from both primary and distant metastatic sites[38].

HER 2 AND TARGETED THERAPY

HER 2 status and the use of molecular targeted therapy in the management and prognostication of the gastric carcinoma is very important. Surgical resection is the primary and recommended surgical treatment, although most patients are diagnosed at an advanced stage in which chemotherapy is the main treatment option. The efficacy of treatment for advanced gastric carcinoma with palliative chemotherapy is poor, so there is great interesting the targeted therapies that are emerging, and several molecular targeting agents are being tested. Unfortunately, Trastuzumab is the only targeted therapy that has a proven survival benefit in gastric carcinoma till date[40]. Trastuzumab

inhibits the HER 2 receptor. Tyrosine kinase activation is effectively blocked by binding the juxtamembrane portion of HER 2 receptor using very two specific antigenic sites of Trastuzumab. Tumor genesis is prevented by Trastuzumab by not allowing HER 2 protein to undergo heterodimerization. HER 2-mediated signaling is inhibited by raising the antibody-dependent cellular cytotoxicity and cleavage of the extracellular domain of HER 2 is also inhibited[41]. In gastric carcinoma patients with overexpression of HER 2, the addition of Trastuzumab cisplatin resulted in better response rate (35%) and stable disease (17%). The efficacy and safety of Trastuzumab were evaluated in HER 2 positive advanced gastric carcinoma by Trastuzumab for Gastric Cancer Trial (ToGA),the largest multicentric trial for targeted therapy HER 2(42). Were randomized to treatment with chemotherapy alone vs. Trastuzumab with 5FU/capecitabine and cisplatin. Preliminary results showed better median survival with the combination regime (13.5 vs. 11.1 months), with a reduction in risk of death (26%). In the pre-planned analysis, HER 2 positive patients (IHC2+/FISH+ or IHC3+) showed a better survival trend with longer survival (16 months) with Trastuzumab and chemotherapy compared with chemotherapy alone (11.8 months)[42].

MATERIALS AND METHODS

STUDY SETTING

This study was conducted in Christian Medical College, Vellore in the department of General pathology on 66 consecutive mucosal biopsies with sufficient tumor and corresponding gastrectomy specimens of gastric adenocarcinoma diagnosed between July 1, 2016, and June 30, 2017. The clinic pathological details were reviewed systematically from the electronic medical work station, and Radiological, and endoscopy details were retrieved wherever possible from the same. The haematoxylin and eosin stained slides were reviewed for classification, grading and staging. Immunostaining for HER 2 was performed on freshly cut sections and the positive staining graded (intensity, pattern and of positive tumor cells). If both mucosal biopsies and resected tissue were available in a case, both sections were placed on the same slide for simultaneous IHC staining. HER 2 expression of the tumor cells was correlated with the anatomical site, classification, staging and grading of a tumor. The data on mucosal biopsies and surgical resections was analyzed separately. Correlation of HER 2 expression on mucosal biopsies versus surgical resections was also performed. Any discordance in matched resection and biopsy specimens was analyzed for heterogeneity. In all HER 2 positive cases, IHC was performed in multiple blocks from all quadrants of the tumor to demonstrate intratumoral heterogeneity.

RESEARCH BUDGET PLAN.

Institutional review board (IRB) Minutes number: **10206**approved our study.

Interdepartmental collaboration between General Pathology, Medical Oncology, General Surgery and Gastroenterology significantly improved the quality of our research. The Institutional Fluid Research grant account number (**22 Z 141**) was used to cover the costs of IHC staining.

SAMPLE SIZE:

The aim of the study the prevalence of HER 2 expression in gastric adenocarcinoma by IHC and to correlate the expression HER 2 with the stage, grade, classification and location of gastric adenocarcinoma. The expression of HER 2 was separately evaluated in mucosal biopsies and surgical resections and in those patients where both were available, the expression was correlated. Patients who had consecutive mucosal biopsies with sufficient tumour and corresponding resected specimens of gastric adenocarcinoma diagnosed in our hospital (resection specimens) from July1st 2016 to July 30th 2017 were recruited into our study. Preliminary analysis showed that the sample size required was approximately 66 cases to meet the objectives of the study.

SAMPLE SIZE CALCULATION:

The sample size was calculated using nMaster 2.0 software. The formula used was

Sample size calculation using prevalence.

n= sample size e= ± level of precision around estimated HER 2 prevalence

p= prevalence of HER 2 over expression

Zα = 1.96 area under the standard normal curve representing

(95% confidence interval.)

1- P= 1 – prevalence of HER 2 expression

Assumptions: Precision = 10.00 %, Prevalence = 22.00 % (TOGA trial)

Population size = infinite

Estimated sample size: n = 66

PARTICIPANTS:

Inclusion criteria

Patients with a confirmed histological diagnosis of gastric carcinoma with sufficient tumour tissue for immunohistochemistry.

Exclusion criteria

Scanty tumour biopsies

GEJ carcinoma patients

Patient's resection performed in another hospital.

Patients histological diagnosed as secondary tumour or distal Squamous cell carcinoma of the esophagus.

BIAS:

Cases were identified only by the number so that all investigators were blinded. All investigators reached agreement on the particular score by consensus to minimize bias.

DATA SOURCES/MEASUREMENT:

Patient data was collected from completed proforma and the institutional electronic work station.

QUANTITATIVE VARIABLES:

The variables analyzed in this study are listed in the proforma (see Annexure 1); Immunohistochemical markers were graded according to the score provided in the literature review.

STATISTICAL ANALYSIS:

The attached proforma was used for data collection. 72 mucosal biopsies and the corresponding resection specimens of gastric carcinoma that met the inclusion criteria were studied. Data entry was done in EPIDATA software and used for statistical analysis. Results on continuous measurements presented as Mean, and SD (Min-Max) and results on absolute measurements are shown in Number (%). The significance was assessed at 5 % level of significance. . Student t-test was used to find the significance of study parameters on the continuous scale between two groups on metric parameters. Chi-square/ Fisher Exact test has been used to determine the significance of study parameters on a categorical scale between two or more groups. All the tests of associations or comparisons were considered significant at 5% (p value≤ 0.05) level of significance. Most of the findings and results are presented using tables and graphs. All statistical Analysis was performed with the help of a professional statistician using SPSS and Microsoft Excel software.

METHODOLOGY

The study utilized 72 matched mucosal biopsies, and the corresponding surgical resections of the same patients who were diagnosed with gastric carcinoma in the Department of Pathology from 1 July 2016to30 June 2017. As soon as the specimen was received from the operation theatre, Specimens were fixed in neutral buffered formalin (10%) See figure. 15 to 20 times the volume of the specimen was used for formalin fixation. For gastric resection specimens, 18- 24 hours was recommended, and the interval between tissue acquisition and fixation was kept as short as possible (< 1 hour). Because of the high probability of gastric heterogeneity, a minimum of 6 gastric biopsy fragments were taken from different areas. The standard grossing procedure was followed. A minimum of four sections through a wall, including tumour borders and adjacent mucosa were submitted for paraffin block preparation and haematoxylin and eosin staining (H&E). Fixation was performed at room temperature (15- 25°C). Processed tissues were embedded in new paraffin. Prolonged incubation in molten paraffin was meticulously avoided as high temperatures can degrade epitopes. Concerning surgical samples, the pathologist would select tissue blocks with the largest area of intestinal differentiation (glandular structures) for HER2 staining. The use of pre diluted VENTANA HER2 (4B5) and corresponding Detection Kits, in combination with a VENTANA automated slide stainer Benchmark XT(See figure10)reduced the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting, and manual reagent application.

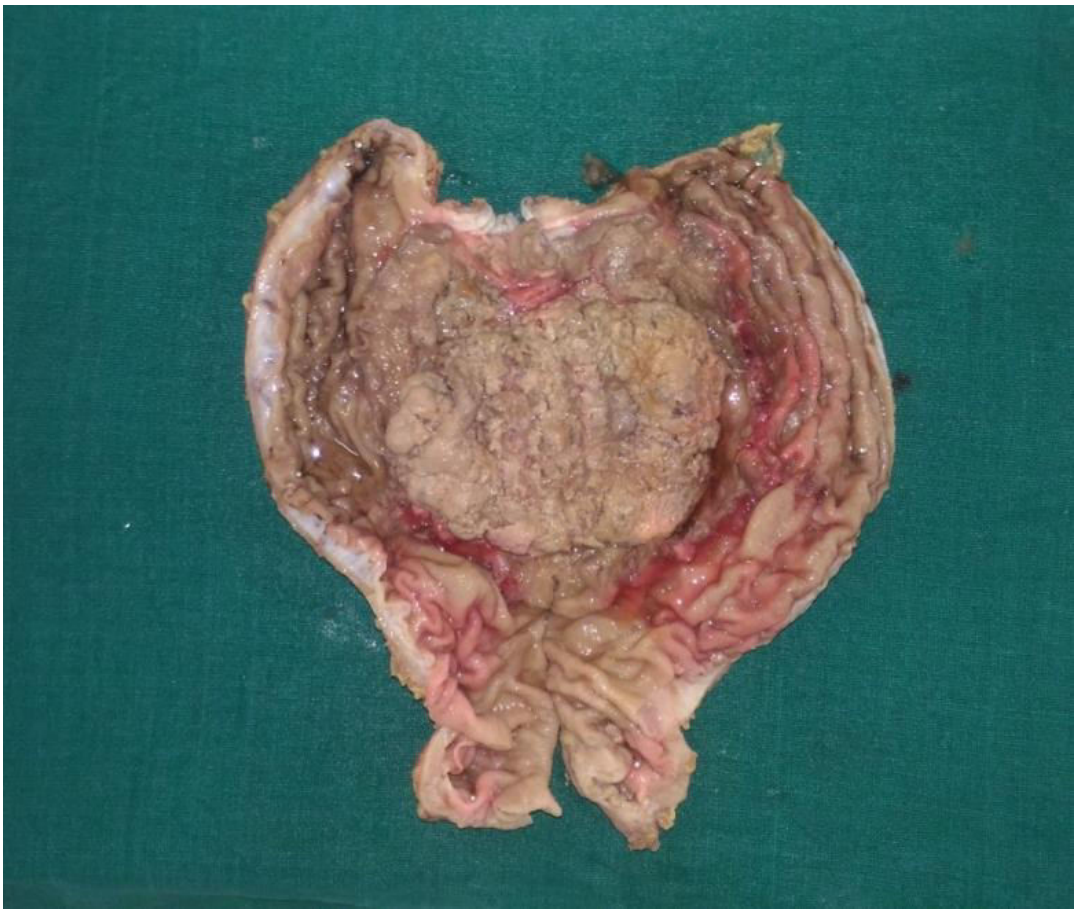

Figure9. Specimen fixation. Total gastrectomy specimen with Fungating growth in the lesser curvature cut opened along, the lesser curvature and fixed with the pin into the wax board before fixing with 10% formalin solution.

Figure 10.Ventana Bench Mark XT.

STUDY ALGORITHM

Mucosal biopsies and resected specimen of Gastric adenocarcinoma cases

Review of all slides and study of tumour characteristics

Selection of representative tissue blocks and run IHC –HER -2 (Ventana Benchmark XT)

Assessment of expression of IHC-HER-2

Analysis and comparison of histopathology with Immunohistochemistry

RESULTS

CLINICAL FEATURES

The study was conducted in a tertiary hospital in south India on a total of 72 cases of gastric adenocarcinoma diagnosed over period of one year from 2016-2017. 49 patients were male (68%), with a sex ratio of 2.1:1. The mean age of patients was 53 years (range 23 years to 82 years, with a standard deviation of 12.53). The mean age of female patients alone was 46 years suggesting that women may have gastric adenocarcinoma stomach at a younger age the highest incidence of gastric carcinoma, both in women and men in our study, was found in the fifth decade of life. Majority of the patients in the study were from West Bengal and Bangladesh. This could be a reflection of the patient population attending our hospital. Patients from Tamil Nadu were the next most frequent. Among the surgical specimens, 55 were distal subtotal gastrectomies, and 17 were total gastrectomies.

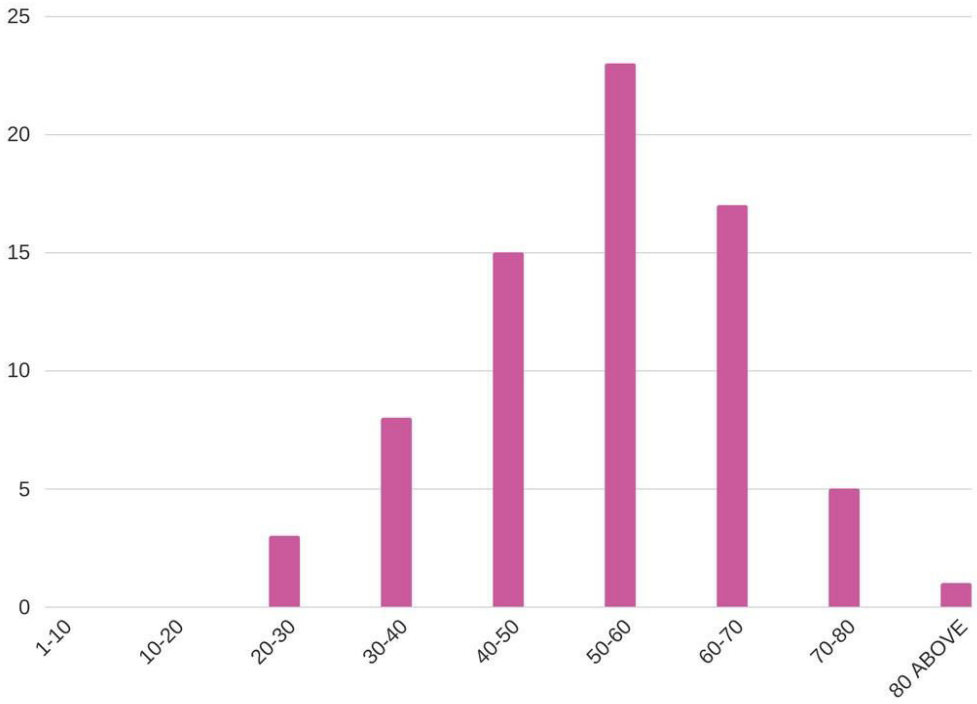

Figure 11.Distribution of patients by age

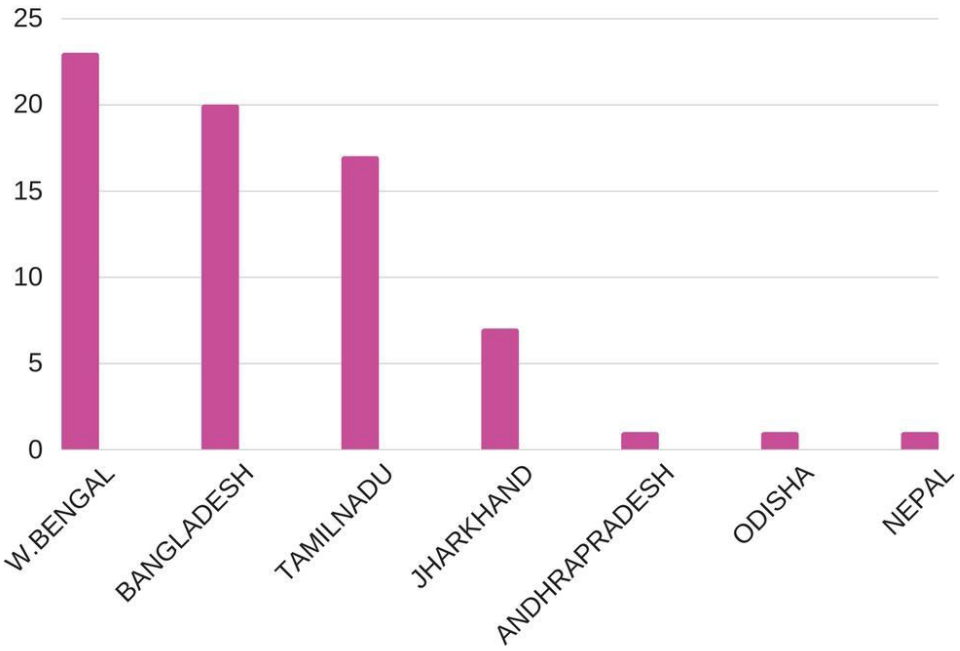

Figure 12.Distribution of patients by place

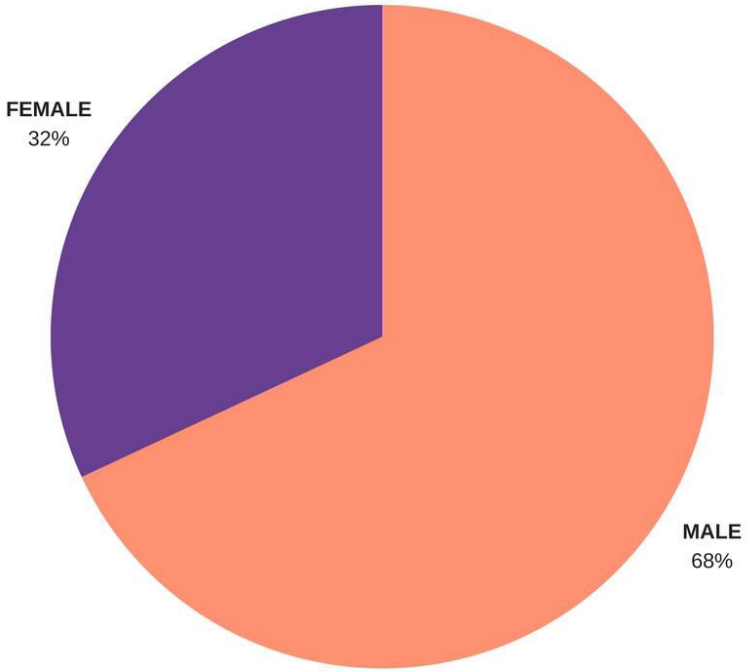

Figure 13.Distribution of patients by sex

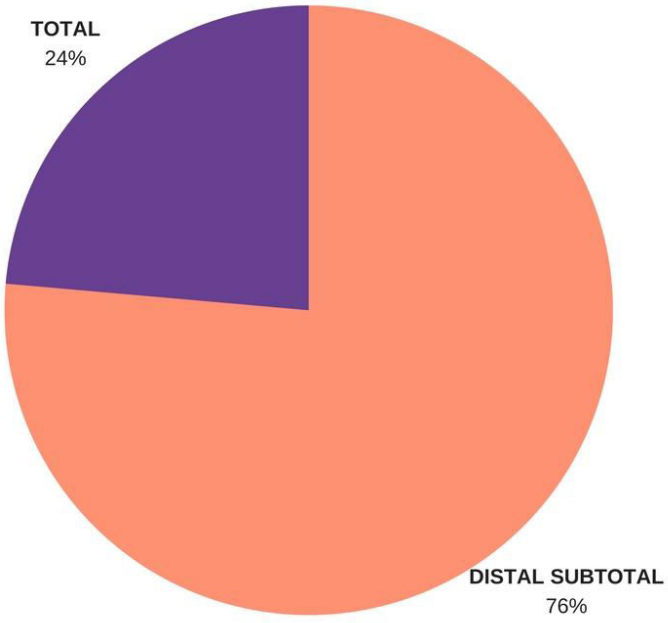

Figure 14.Distribution of patients by Surgery

PATHOLOGICAL FEATURES

The following pathological parameters were evaluated.

TUMOR LOCATION

In 58(80.5%) cases, the tumor was located in antrum/pylorus. In 3 cases (4%) the tumor was found in body of the stomach, in 9cases (12.5%) in the fundus/cardia of the stomach, and in 2 (3%) cases carcinoma involved the whole stomach. 46 (63.89%) exhibited a circumferential growth pattern, 21 cases (29.17%) were located in the lesser curvature of the stomach, and 5 cases (7%) in the greater curvature of the stomach.

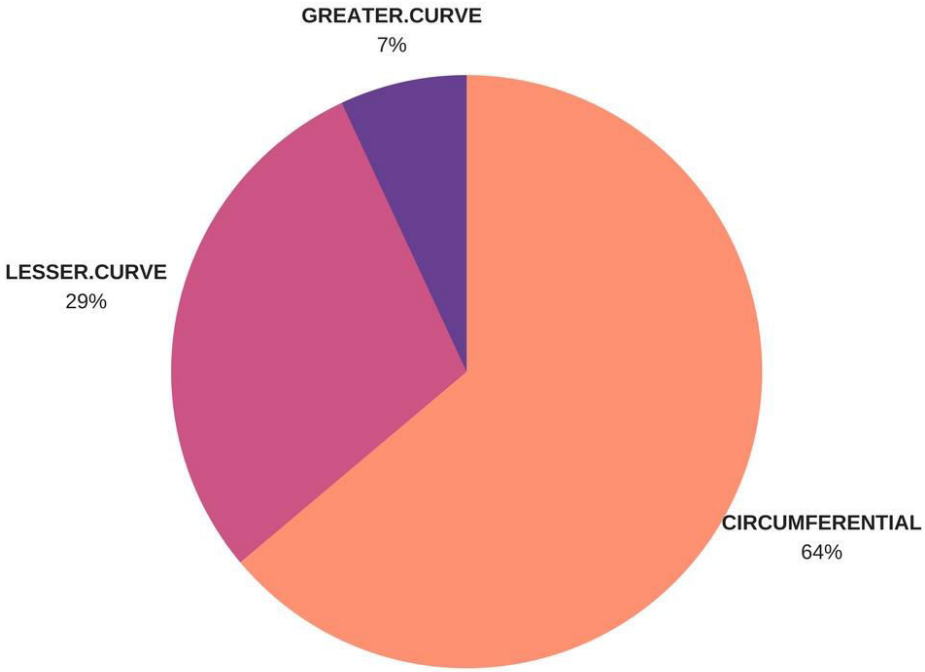

Figure 15.Distribution of cases of gastric cancer depending on curvature

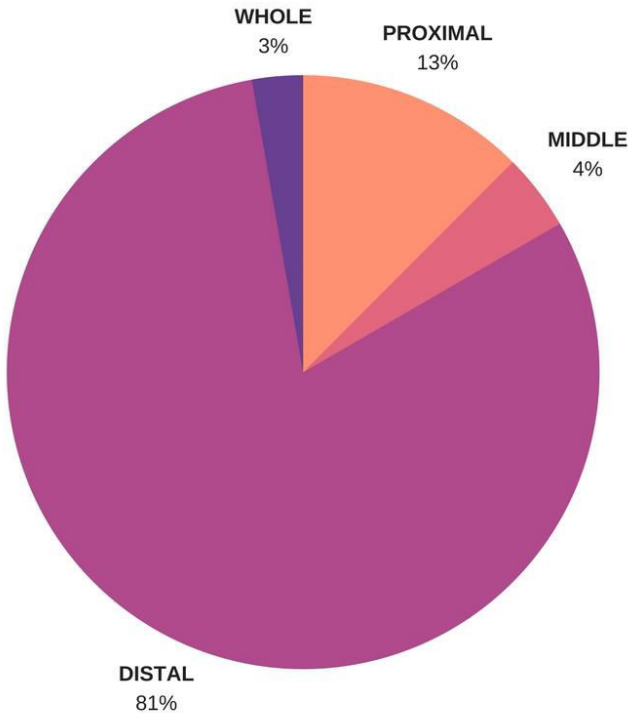

Figure 16.Distribution of cases of gastric cancer depending on location

CLASSIFICATION

BORMANN CLASSIFICATION: The most common form of advanced gastric carcinomas was Bormann type III (Ulcerated) observed in 33 patients (45%), followed by type II in 21 cases (29%). (SeeFigure17).

WHO 2010 CLASSIFICATION: According to the WHO classification, of the 72 cases of gastric carcinomas, 25(34.7%) cases were classified as tubular adenocarcinoma, 27 cases (37.5%) as poorly cohesive carcinomas and 6 (8, 3%) as signet ring cell carcinoma. 12 cases (16.7%) were classified as mixed adenocarcinoma. 1 (1.3%) cases were classified as mucinous adenocarcinoma. 1 (1.3%) cases classified as lymphoid stroma variant adenocarcinoma (see Figure18).

LAUREN CLASSIFICATION: Based on the Lauren classification of gastric carcinoma, 26 were intestinal type carcinomas (36.1%), 44 diffuse type carcinomas were (61.1%). A tiny percentage of gastric carcinomas, 2% (3 cases), were indeterminate. (See Figure19).

MODIFIED LAUREN CLASSIFICATION: Based on the Modified Lauren classification of gastric carcinoma, 6wereproximal non-diffuse carcinomas (PND) (8.33%), 45diffuse carcinomas (D) (62.5%) and 21 distal non diffuse (DND) type carcinomas (29.17%). (See Figure20).

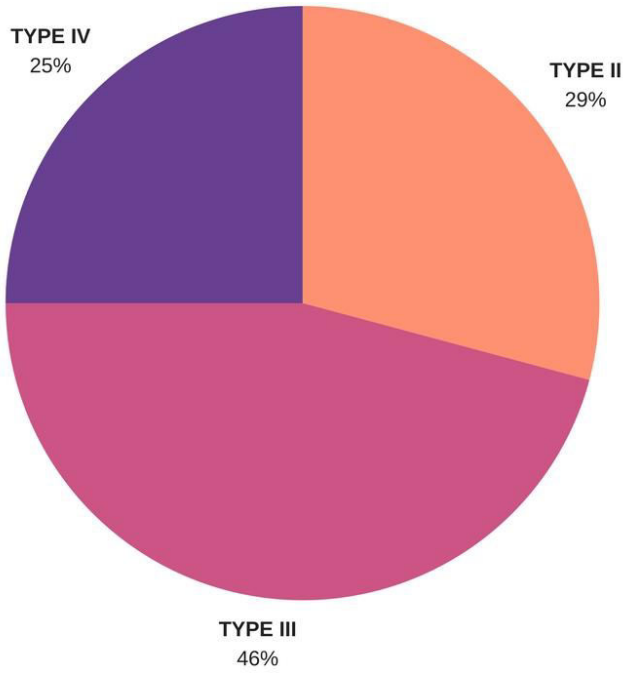

Figure 17.Distribution of cases according to Bormann classification

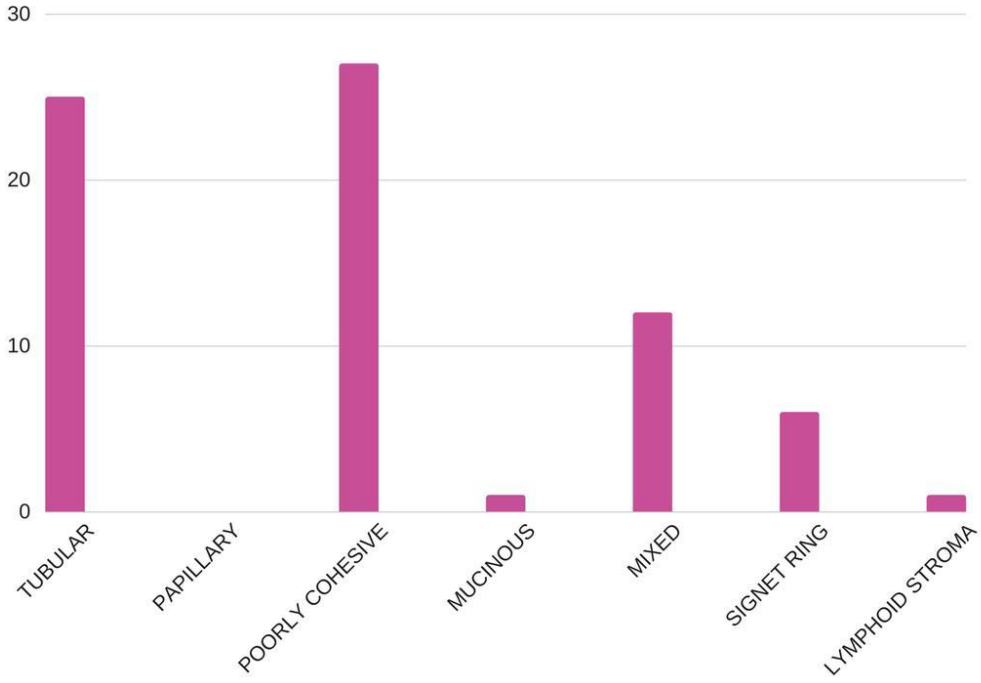

Figure 18.Distribution of cases according to WHO classification

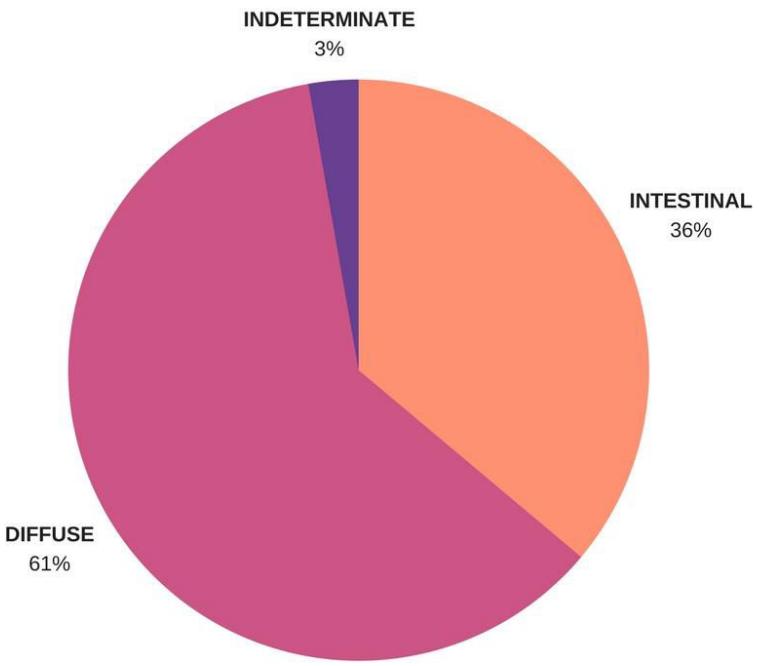

Figure 19.Distribution of cases according to Lauren classification.

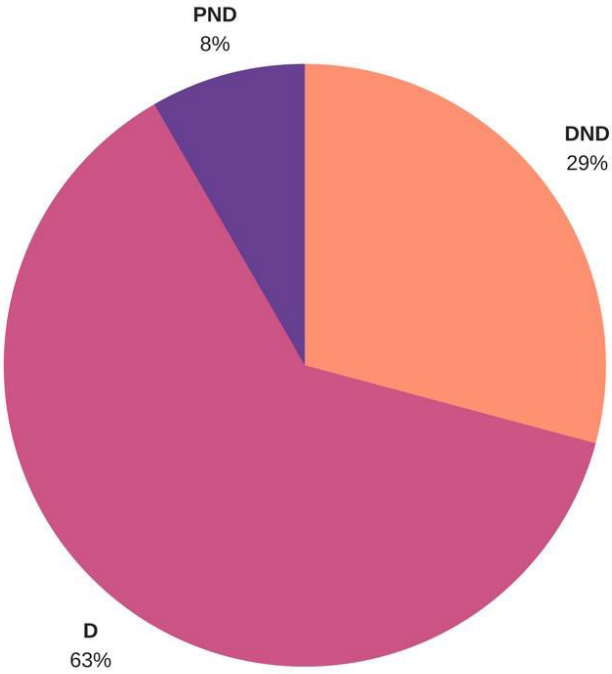

Figure 20.Distribution of cases according to Modified Lauren classification

TUMOR GRADE

1(1.39%) was a well-differentiated adenocarcinoma (G1), 29 (40.3%) - moderately differentiated adenocarcinoma (G2) and 42 (58.3%) - poorly differentiatedadenocarcinomaG3 (see Figure21)

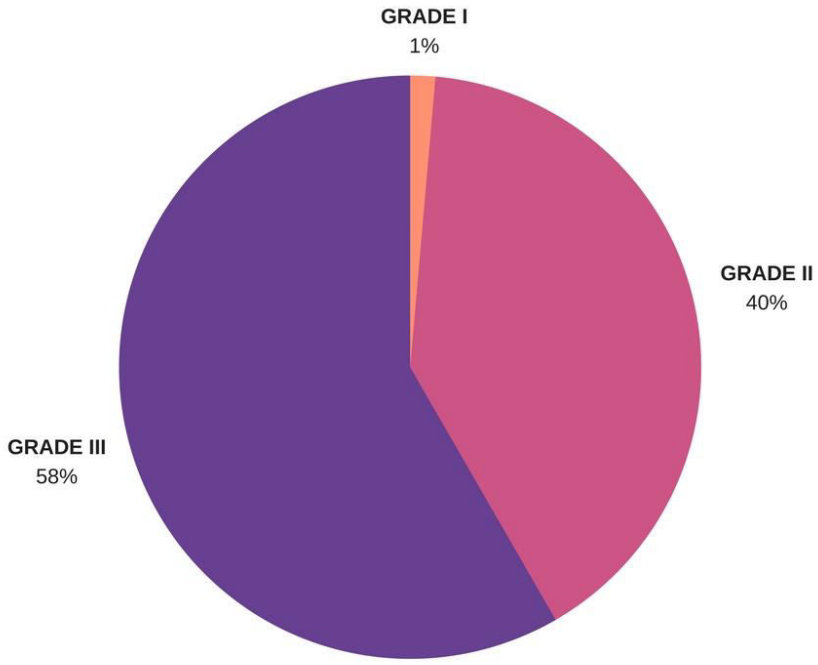

Figure 21.Distribution of cases according to tumor grade

DEPTH OF INVASION (T)

Carcinoma was limited to the mucosa in 3 patients (4.17%). 2 (2.8%) of cases were confined to the sub mucosa. In seven there was muscular coat invasion (9.7%). In 22cases, invasion was limited to the subserosal connective tissue (30.6%) and in 38

(52.8%) cases, adenocarcinoma invading the gastric wall entirely, reaching the peri gastric fat. (See Figure 22).

LYMPH NODE AND DISTANT METASTASIS

Lymph node metastases were present in 60 cases (83 %%). 1 case had liver metastasis. (See Figure 23&24).

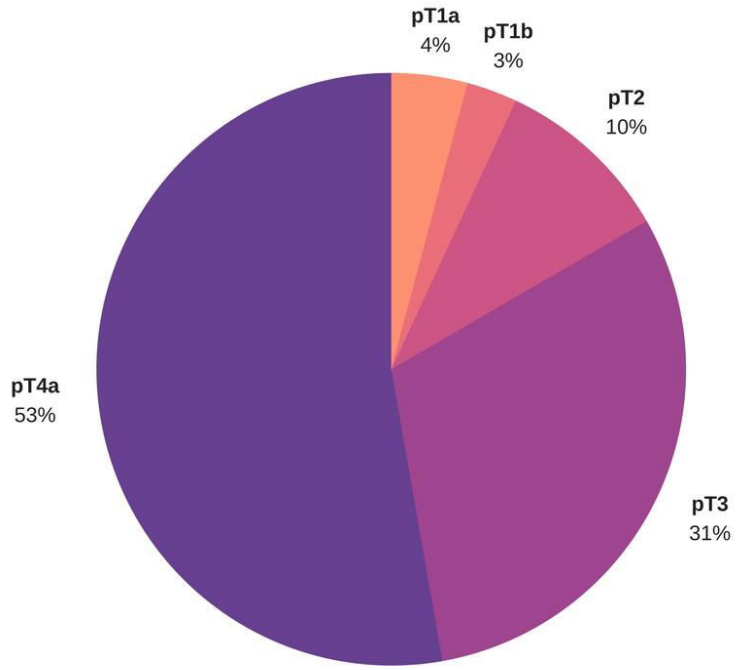

Figure 22.Distribution of cases according to depth of invasion

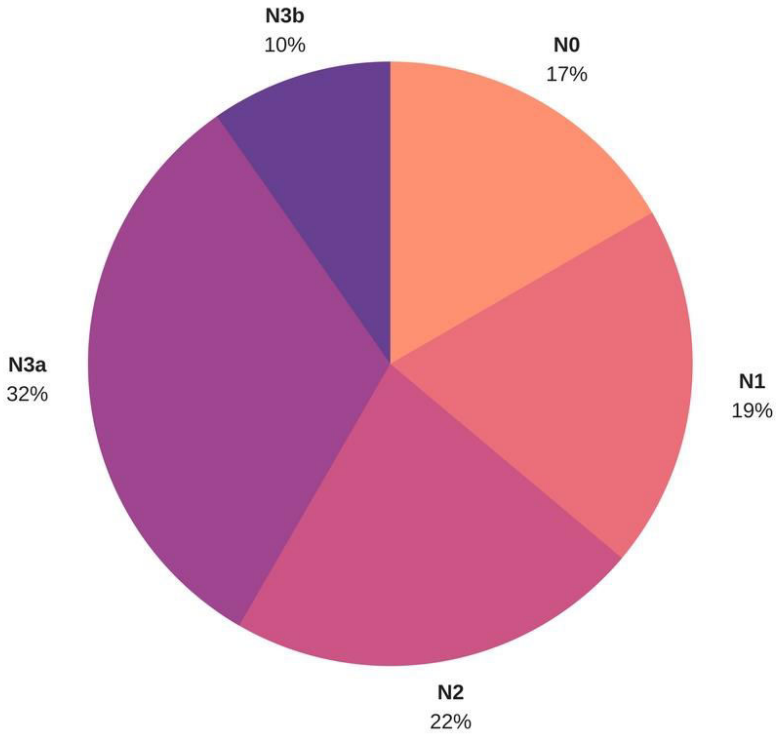

Figure 23.Distribution of cases according to regional lymph node metastasis

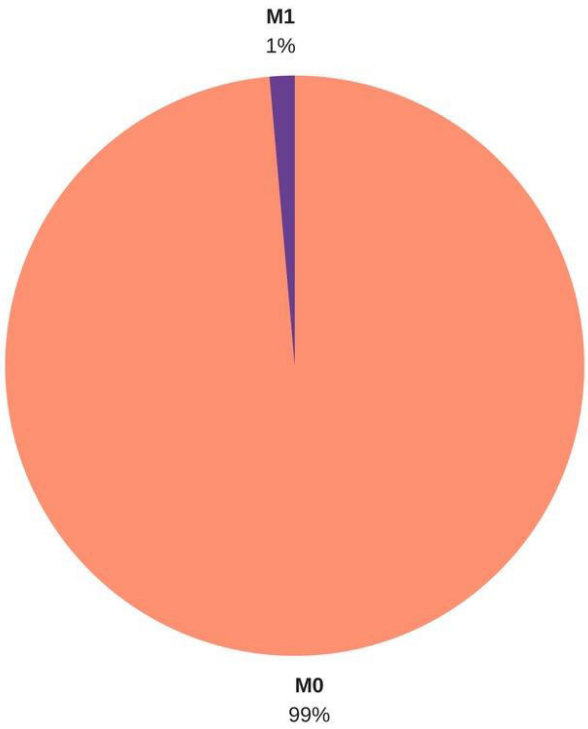

Figure 24.Distribution of cases according to distant metastasis

STAGING

The highest numbers of cases were stage III disease in which curative oncological treatment was difficult to achieve. 48tumours were in stage III (66.8 %), 16 in stage II (22.1%) (See Figure25).

LYMPHO VASCULAR AND PERINEURAL INVASION

Lymph vascular invasion and Perineural invasion were found in28 cases (38.9%), and in44 cases (61.1%) respectively. (See Figure 26&27).

PRECANCEROUS CONDITION

Gastric mucosa adjacent to the tumor was evaluated for various additional findings. 11 out of 72 (15.28 %) patients showed H.pylori infection with associated gastritis. 5 (6.94%) cases showed Intestinal metaplasia. (See Figure 28).

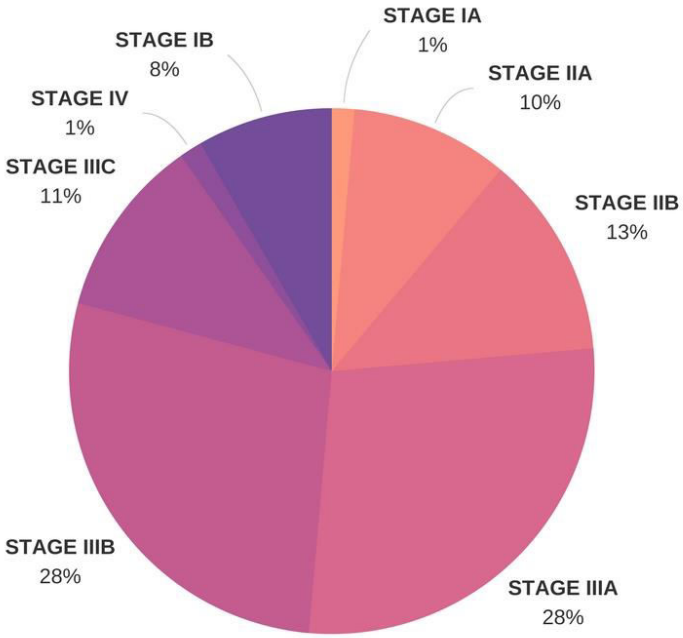

Figure 25.Distribution of cases according to pTNM stage

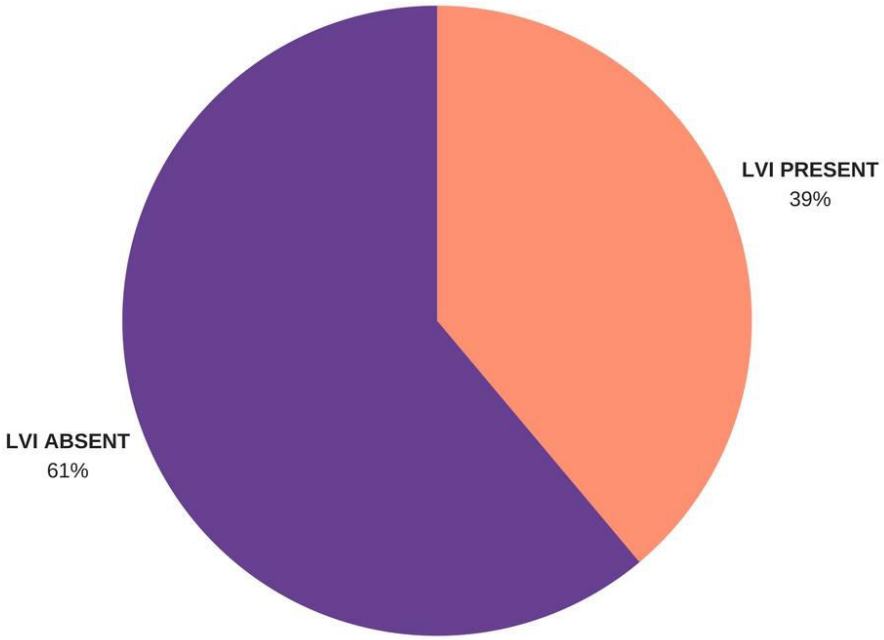

Figure 26.Distribution of cases according to Lympho vascular invasion

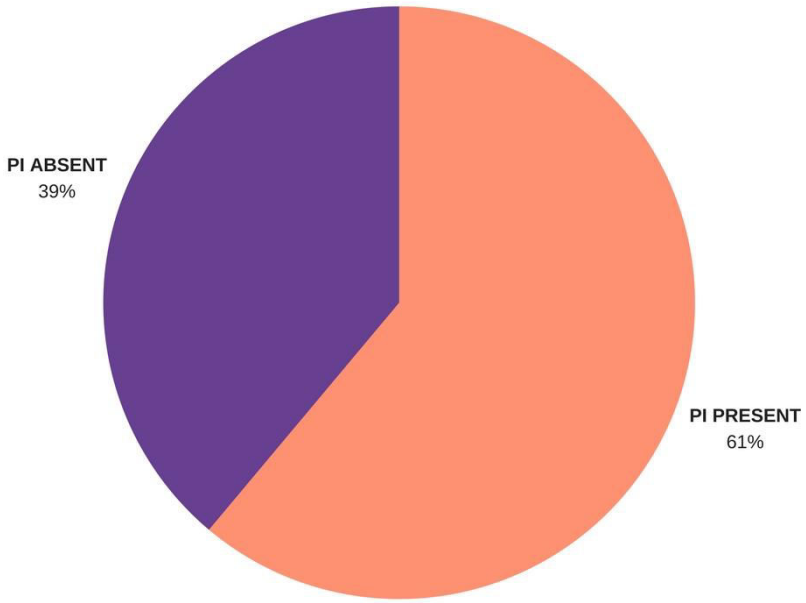

Figure 27.Distribution of cases according to Perineural invasion

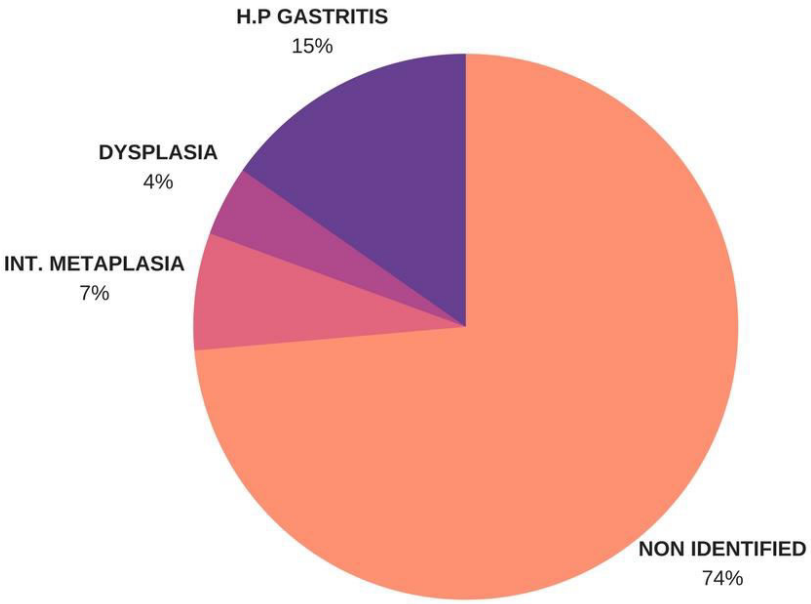

Figure 28.Distribution of cases according to precancerous condition

PROXIMAL MARGIN

Proximal Margin was involved in 3 (4%) of the resected specimens.

DISTAL MARGIN

Distal Margin was involved in 6 (8%) of the cases. 1 patient had involvement of both the margins.

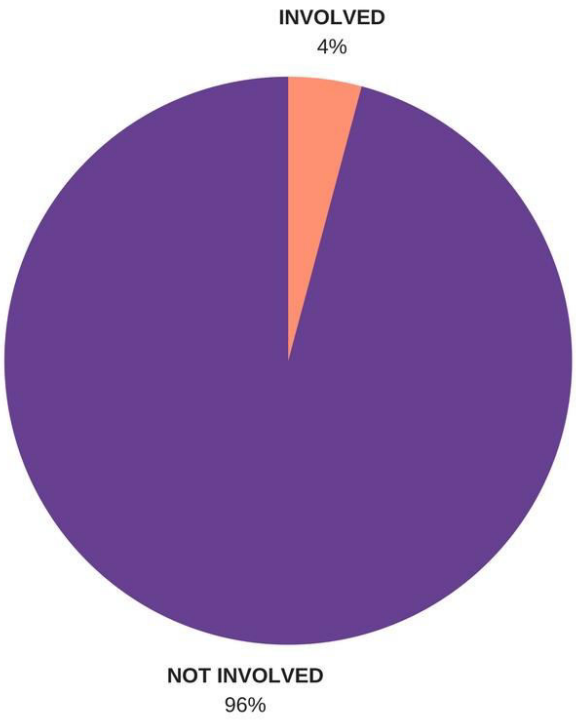

Figure 29.Proximal margin involvement

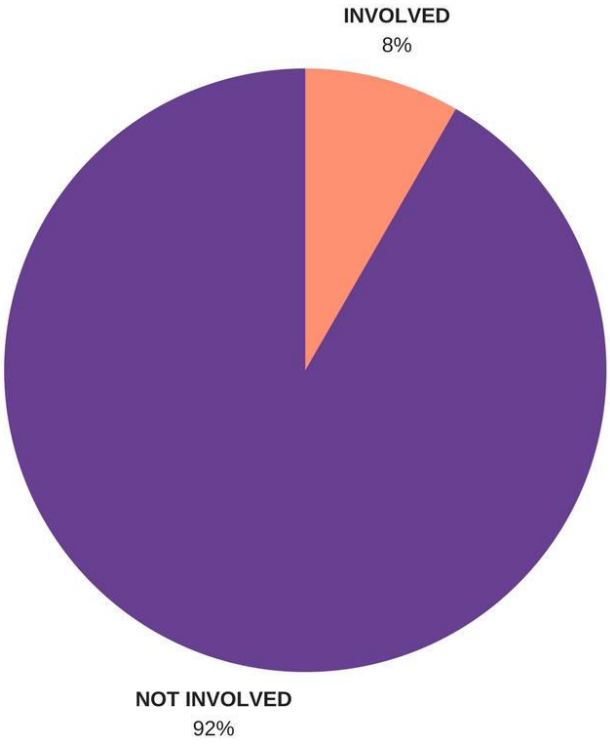

Figure 30.Distal margin involvement

NEOADJUVANT THERAPY:

14 of 72 patients (19.4 %) had received neoadjuvant chemotherapy, and Tumour regression score evaluated. Out of these 14 cases, 5 (6.9) cases showed a poor response, 3(4.1%) cases showed minimal response and 6 cases showed a moderate response.

(See Figure31).

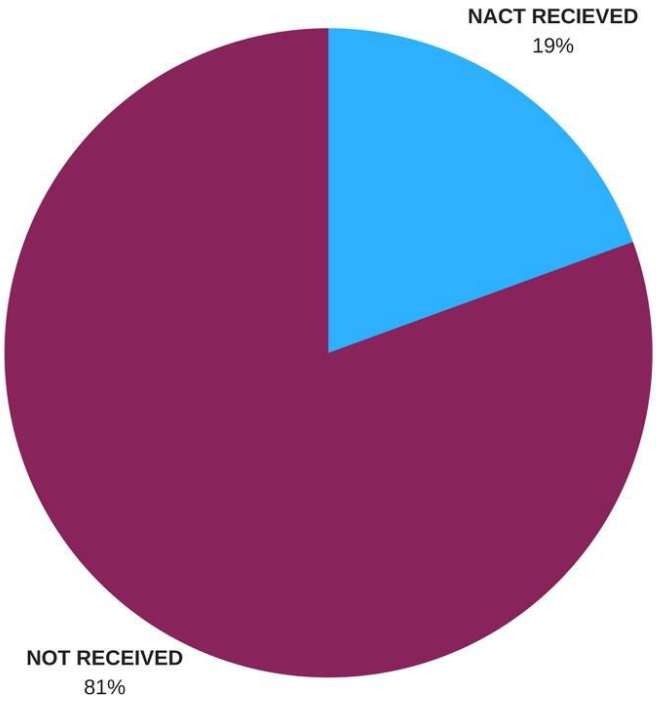

Figure 31.Distribution of cases according to neoadjuvant therapy cases

HER 2 IHC STUDY

HER2 AND DEMOGRAPHY

On Immunohistochemical analysis, eight cases were positive (3+; 11.1 %) for HER2 protein over expression and 64 cases were negative (88.9 %). In this study, there were no equivocal cases (2+). IHC HER 2 expression was found more often in men, but the correlation between HER2 expression and patients' gender was insignificant (see table 6).Correlation between HER2 expression and patient's geographical location was also found to be insignificant. (See table 6).

HER 2 EXPRESSION AND CLASSIFICATIONS

There was a positive association between HER2 expression and tumours classified as WHO mixed adenocarcinoma (5/12 (41.7%), P=0.009).Bormann ulcerated variant also showed increased HER 2 positivity, but this was not statistically significant. Laurens, Modified Lauren and Bormann classification parameters in our study did not show significant association with HER2 expression. For detailed comparison see (table 7).

Table 6.Association between HER2 status and clinic statistical parameters

	Overall n=72(100%)	HER2 positive 8(11%)	HER2 Negative 64(89%)	P value (Significant<0.05%)
Age				1
<50 yrs	26(36.11)	3(37.5)	23(35.94)	
>50 yrs	46(63.89)	5(62.5)	41(64.06)	
Gender				0.422
Male	49 (68)	7(87.5)	42(65.62)	
Female	23(32)	1(12.5)	22(34.38)	
Tumor topography				0.173
Distal antrum	58(80.56)	5(62.5)	53(82.81)	
Body	3(4.17)	0(0.0)	3(4.69)	
fundus/cardia	9(12.5)	3(37.5)	6(9.38)	
whole stomach	2(2.77)	0(0.0)	2(3.13)	
Tumour curvature				0.836
Lesser	21(29.17)	3(37.5)	18(28.13)	
Greater	5(6.94)	0(0.0)	5(7.81)	
Circumferential	46(63.89)	5(62.5)	41(64.06)	
Place				0.973
Bangladesh	23(31.94)	3(37.5)	17(26.56)	
West Bengal	23(31.94)	3(37.5)	20(31.25)	
Tamil nadu	17(23.61)	2(25.0)	15(23.44)	
Jharkhand	7(9.72)	0(0.0)	7(10.94)	
A.Pradesh	3(4.17)	0(0.0)	3(4.69)	
Nepal	1(1.39)	0(0.0)	1(1.56)	
Odisha	1(1.39)	0(0.0)	1(1.56)	

Table 7.Association between HER2 status and classifications

	Overall n=72(100%)	HER2 positive 8(11%)	HER2 negative 64(89%)	P value (Significant<0.05%)
Bormann				0.637
Type I	0(0.0)	0(0.0)	0(0.0)	
Type II	21(29.17)	2(25.0)	19(29.69)	
Type III	33(45.83)	5(62.5)	28(42.75)	
Type IV	18(25.00)	1(12.5)	17(26.56)	
Laurens				0.572
Intestinal	26(36.11)	4(50.0)	22(34.38)	
Diffuse	44(61.11)	4(50.0)	40(62.5)	
Indeterminate	2(2.78)	0(0.0)	2(3.13)	
Modified Laurens				0.593
PND	6(8.33)	1(12.5)	5(7.81)	
D	45(62.5)	4(50.0)	41(64.06)	
DND	21(29.17)	3(37.5)	18(28.13)	
WHO(2010)				**0.009**
Tubular	25(34.72)	3(37.5)	22(34.38)	
Papillary	0(0.0)	0(0.0)	0(0.0)	
Poorly cohesive	27(37.5)	0(0.0)	27(42.49)	
Signet ring cell	6(8.33)	0(0.0)	6(9.38)	
Mucinous	1(1.39)	0(0.0)	1(1.56)	
Mixed	12(16.67)	5(62.5)	7(10.94)	
Lymphoid stroma	1(1.39)	0(0.0)	1(1.56)	

HER 2 AND HISTOPATHOLOGY

Five of 29 moderately differentiated Tumours showed HER2 positivity. The minimum and maximum tumour size ranged between 7 mm and 120 mm (average 49.86 mm). When HER2 protein expression correlated with tumour grade, moderately differentiated tumours were found to be the most standard grade with HER2 expression, but this was not statistically significant (P= 0.34). Correlation between HER2 protein over expression and depth of invasion, lymph node metastasis, and distant metastasis not were found to be significant. There were no IHC 2+ cases. Therefore, cases with a final HER2positive status were exclusively composed of IHC 3+ Tumors and comprised 11.1 % of all gastric carcinoma s in this series. For detailed comparison (see table8).

Table 8.Association between HER2 status and histopatholgical parameters

	Overall n=72(100%)	HER2 positive 8(11%)	HER2 negative 64(89%)	P value (Significant<0.05%)
Tumour grade				0.34
Grade I	1(1.39)	0(0.0)	1(1.56)	
Grade II	29(40.28)	5(62.5)	24(37.5)	
Grade III	42(58.33)	3(37.5)	39(60.94)	
Depth of invasion				0.53
pT1a	3(4.17)	0(0.0)	3(4.69)	
pT1b	2(2.78)	0(0.0)	2(3.13)	
pT2	7(9.72)	2(25.0)	5(7.81)	
pT3	22(30.56)	5(62.5)	17(26.56)	
pT4a	38(52.78)	1(12.5)	37(57.81)	
pT4b	0(0.0)			
Regional L.N				0.859
pN1	12(16.67)	1(12.5)	11(17.19)	
pN2	14(19.44)	1(12.5)	13(20.31)	
pN3a	16(22.22)	2(25.0)	14(21.88)	
pN3b	23(31.94)	4(50.0)	19(29.69)	
Distant metastasis				1
pM0	71(98.61)	8(100.0)	63(98.44)	
pM1	1(1.39)	0(0.0)	1(1.56)	
TNM Staging				0.795
Stage IA	1(1.39)	0(0.0)	1(1.56)	
Stage IB	6(8.33)	1(12.5)	5(7.81)	
Stage IIA	7(9.72)	0(0.0)	7(10.94)	
Stage IIB	9(12.5)	1(12.5)	8(12.5)	
Stage IIIA	20(27.78)	2(25.0)	18(2813)	
Stage IIIB	20(27.78)	4(50.0)	16(25.0)	
Stage IIIC	8(11.11)	0(0.0)	8(12.5)	
Stage IV	1(1.39)	0(0.0)	1(1.56)	
Proximal margin				0.301
Negative	69(95.83)	7(87.5)	62(96.88)	
Positive	3(4.17)	1(12.5)	2(3.13)	
Distal margin				0.52
Negative	66(91.67)	7(87.5)	59(92.19)	
Positive	6(8.33)	1(12.5)	5(7.81)	

Table 9.Association between HER2 status and histopatholgical parameters (contd)

	Overall n=72(100%)	HER2 positive 8(11%)	HER2 negative 64(89%)	P value (Significant<0.05%)
Lymphovascular invasion				**0.049**
Positive	28(38.89)	6(75.0)	22(34.38)	
Negative	44(61.11)	2(25.0)	42(65.63)	
Perineural invasion				0.248
Positive	44(61.11)	3(37.5)	41(64.06)	
Negative	28(38.89)	5(62.5)	23(35.94)	
Precancerous condition				
Non identified	53(73.61)	6(75.0)	47(73.44)	0.443
H. pylori gastritis	11(15.28)	1(12.5)	10(15.63)	
Intestinal metaplasia	5(7.81)	0(0.0)	5(7.81)	
Dysplasia	3(4.17)	1(12.5)	2(3.13)	
Treatment effect				0.34
Grade 0	0(0.0)	0(0.0)	0(0.0)	
Grade 1	1(1.39)	0(0.0)	1(1.56)	
Grade 2	29(40.28)	5(62.5)	24(37.5)	
Grade 3	42(58.33)	3(37.5)	39(60.94)	

HER 2 COMPARISON BETWEEN BIOPSIES AND RESECTION

HER 2 positive status between matched mucosal biopsy and surgical resections shown in the table 10and 11). 66 cases (91.9 %) displayed complete concordance between IHC results on mucosal biopsies and corresponding resection specimens (64 IHC 0/1+ cases and 2 IHC 2/3+ cases). Among the 8 HER 2 positive (3 +) cases, 4 cases Showed good concordance between Resection and mucosal biopsies. Other 4 cases exhibited variable results on endoscopic biopsies and surgical blocks. Two cases show positive shift which means, mucosal biopsy IHC displayed a HER 2 score 0 and the Resection specimen showed IHC positivity (3+). Other two cases revealed a negative shift which means mucosal biopsies showed HER 2 positivity with IHC score 3+ and were HER 2 negative for the matched resection blocks. The negative shift could have mainly been due to the chemo therapeutic effect.

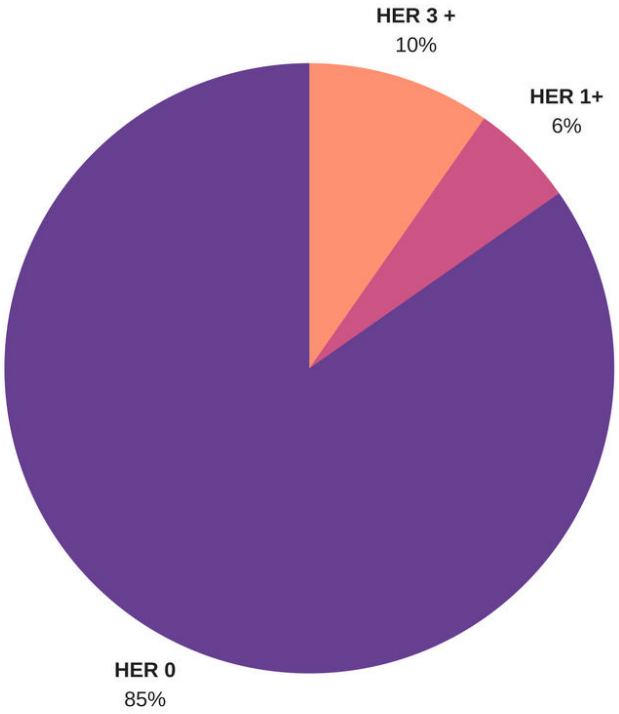

Figure 32.Distribution of cases according to Resection HER 2 status

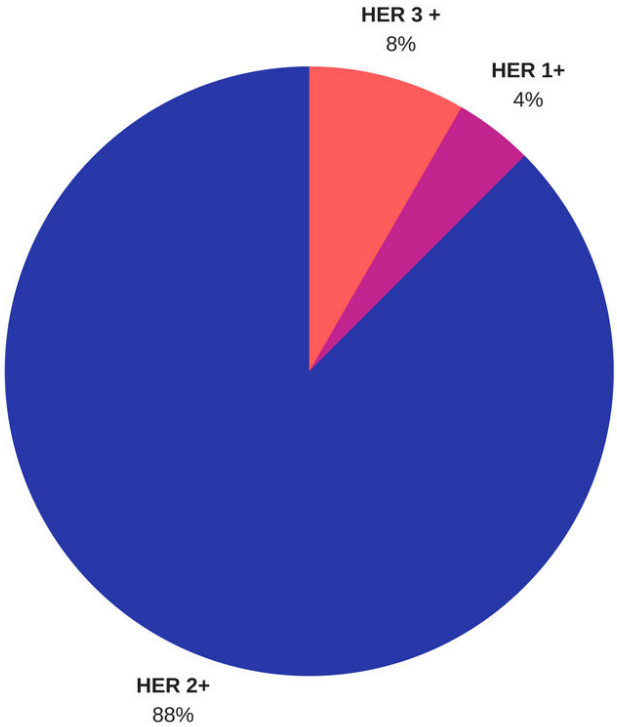

Figure 33.Distribution of cases according to Biopsy HER 2 status

Table 10. HER 2 IHC status

	HER 0	HER 1+	HER 2+	HER 3+	POSITIVE	NEGATIVE	HER %
RESECTION	61	4	0	7	7	65	9.72%
BIOPSY	63	3	0	6	6	66	8.33%

Table 11.Concordance of HER2 Status by IHC in Biopsy and Surgical Samples

Biopsy Samples	Surgical Samples			NPV (%)	PPV (%)
	IHC 0 to 1+	IHC 2+/ 3+	Total		
IHC 0 to 1+	64(98.46)	2(28.57)	66(91.67)	98.462%	-
IHC 2+ to 3+	1(1.54)	5(71.43)	6(8.33)	-	62.5%
Concordance rate: 95.83					

NPV: negative predictive value. PPV: positive predictive value.

HER 2 STATUS AND INTRATUMORAL HETEROGENEITY

Intra tumoral heterogeneity evaluated in the surgical specimens only. All 60 HER 2 negative cases in the resection block were homogeneously negative. 12 cases showed positive immunostaining (IHC score 1+, 2+, or 3+). 4 cases showed a complex pattern and all of these scored 3+. Out of 8 HER 2positive cases3 cases had given adjuvant chemotherapy. In all HER 2 positive (3+) cases, sections from two separate surgical

paraffin blocks evaluated for staining. NACT resection samples excluded from assessing the heterogeneity. Complete correspondence between blocks seen in 2 cases and the remaining 3 cases comparison between blocks provided different results. Intratumoral heterogeneity and WHO mixed adenocarcinoma show the significant relationship, and in these cases neoplastic glandular areas stained strongly with HER2. Two cases showed homogeneous staining pattern (3 +), and both cases were Tubular variants of the WHO classification (2010), and of the intestinal type (Laurens) with tumour grade II. All 3 Cases displaying intratumoral heterogeneity were mixed adenocarcinoma (WHO 2010) with tumour Grade II and Grade III (See Table 12)

Table 12.Tumour heterogeneity in HER 2(3+) positive cases

Cases scenarios	WHO 2010	NACT	HER2 positive biopsy	HER2 positive resection	Tumour heterogeneity
Case 5	Mixed	Non NAC	0	3+	present
Case 15	Tubular	Non NAC	3+	3+	Absent
Case 35	Mixed	Non NAC	3+	3+	present
Case 59	Tubular	Non NAC	3+	3+	Absent
Case 60	Mixed	Non NAC	3+	3+	present
Case 62	Tubular	NAC	3+	0	NA
Case 64	Mixed	NAC	0	3+	NA
Case 68	Tubular	NAC	3+	0	NA

NA: Not applicable (Patients who received neo adjuvant chemotherapy (NAC) excluded)

DISCUSSION

In this study, we compared HER2 expression in matched mucosal biopsies and surgical specimens of patients with gastric adenocarcinoma. We also correlated HER 2 expression with important prognostic pathological parameters, the influence of non-Trastuzumab containing neo adjuvant chemotherapy (NAC) on this expression and studied tumour heterogeneity among the specimens with HER 2 positivity.

PREVALENCE

We found a HER2-positive status in11.1 % of all samples. The TOGA study represents one of the largest sets of HER2 testing data in gastric carcinoma samples, obtained from patients from 24 different countries in Asia, Europe and Latin America. The HER2 positivity rate in the ToGA study was 22% (810 of 3,665 patients) and is twice the prevalence we found in our series. However when the IHC results alone were considered only 398 cases (10.9 %) were HER2 positive (3+). In our study, since there were no tumours with IHC 2+ score, the final HER2 positivity prevalence was11.1 %, matching with the IHC findings of the international TOGA trial.

The prevalence of HER 2 is known to vary between populations. The present study exclusively examined gastric carcinoma and the expression of HER2 by IHC. Gastric adenocarcinoma reported a broad range of HER 2 over expression ranging from 7.6% to 44.2 %(seeTable.13). This variation may be due to various factors such as methodology,

different primary antibody clones, various IHC protocols, various IHC scoring systems, standard operating protocol, and differences in the reporting protocol, tumour heterogeneity and population heterogeneity studied. In the current study, a HER2 protein over expression of 11.1 % correlates with the findings of other international global studies and similar Indian studies. (See Table13), Sekaran et al. in a study conducted in Hyderabad, India observed 44.2 % HER2 positivity. Although genetic variation, dietary habits or precancerous conditions may affect HER 2 positivity rates in gastric carcinoma around the world, the exact relationship between these factors and HER 2 expression is still unknown. The Ventana pathway using rabbit monoclonal antibody (4B5) was used to perform IHC in the TOGA trial and our current study. When compared to 4b5 clone, other tests (eg: HercepTest) report a lower sensitivity for the detection of HER2 protein expression. Data on HER2 expression in Indian patients with gastric carcinoma is insufficient, and there is a wide variation in published results.

AGE AND GENDER

Most studies show no association between HER 2 expression and age or sex of the patient, except for the studies by Matsusaka et al. and FAN et al. that showed a statistically significant correlation(P=0.001) with the male gender. For further detailed comparison between similar studies global population and Indian population see (Table14)

Table 13.HER 2 studies with global prevalence

Study	Place and year	Patients	HER2IHC in %
Bang YJ et al [42]	ToGA trial(2010)	3,665	16.6%
Cho J et al [43]	S. Korea (2013)	2,798	7.3%
Shan L et al [44]	China (2013)	1,463	9.8%
Matsusaka et al[45]	Japan (2015)	1,461	15.6%
Cappellesso et al[46]	Europe (2015)	1,040	11.0%
Phan etal[47]	Vietnam(2017)	208	24.5%
Yoshida et al[48]	Japan (2014)	207	17 %
Hofmann et al[31]	Germany (2009)	178	10.7%
Laboissiere et al[49]	Brazil (2015)	124	10.5%
Hadi etal[50]	Egypt (2012)	85	14.2%
Gharsalli T etal[51]	Tunisia (2017)	84	10.5%
Ogun et al[52]	Nigeria(2014)	36	11 %
Sekaran etal[30]	India(2012)	52	44.2%
Rajagopal etal[53]	India(2015)	60	26.7%
Aditi etal[54]	India(2015)	58	27.6%
Gupta etal[55]	India(2017)	110	24.5%
Current study	India (2017)	72	11.1%

Table 14.HER 2 expression comparisons with age and gender

Study	Gender			Age		
	Male	Female	P value	Younger	Older	P value
Shan L[44]	109/1104(9.9%)	34/359(9.5%)	0.333	63/780(8.1%)	80/683(11.7%)	0.12
Laboissiere[49]	7/64 (10.9%)	6/60 (10%)	0.865	9/82(10.9%)	4/42(9.5%)	0.865
Fan[56]	73/713(10.3%)	18/244(7.4%)	**0.001**	66/682(9.7%)	25/295(8.5%)	0.418
Current study	7/49(14.2%)	1/23(4.3%)	0.422	3/26(11.5%)	5/46(10.9%)	1
Aditi[54]	12/43(27.9%)	4/15(26.7%)	0.103	9/36(25%)	7/22(31.8%)	0.781
Gupta[55]	23/77(29.9%)	4/33(12.1%)	0.116	12/64(18.8%)	15/46(32.6%)	0.269
Rajagopal[53]	11/36(30.56%)	5/24(20.8%)	0.056	*	*	*
Sekaran[30]	16/34(47%)	7/18 (46.7%)	0.769	9/19(32.6%)	14/33(42.4%)	0.777

* Not evaluated in the particular study

TUMOR GEOGRAPHY

Different studies have reported conflicting results regarding tumour location and Her2 expression (see Table 15). Many authors and the current study showed no significant association between Her2 positivity and tumor topography. However the GERCOR study and Matsusaka et al. reported a significant association of Her2 positivity with proximal tumours. Gastric carcinoma in the Indian population tends to arise predominantly in the antrum especially when associated with precancerous conditions especially Helicobacter pylori chronic infection, tobacco and various dietary factors (seeTable15). The tumors of

the current study were mainly distal gastric carcinoma (58 cases out of 72). Our population is expected to have a lower HER 2 positivity rates. HER 2 expression did not show any association with the location along the gastric curvatures in our study. For further detailed comparison between similar studies global population and Indian population (seeTable15 and 16).

Table 15.HER 2 expression comparisons with Tumor geography

Study	Location			P value
	Proximal	Middle	Distal	
GERCOR *etal*[57]	21/105(20%)	6/571(10.5%)	5/56(8.9%)	**0.017**
Laboissiere *etal*[49]	3/24(12.5%)	*	10/100(10%)	0.720
Hadi *etal*[50]	1/14(7.1%)	*	6/37(16.2%)	0.575
Current study	3/9(33.3%)	0/3(0%)	5/58(8.6%)	0.173
Gupta *etal*[55]	17/56(30.3%)	*	9/47(19.1%)	0.548
Sekaran *etal*[30]	4/10(40%)	*	9/47(19.1%)	1

* Not evaluated in the particular study

Table 16.HER 2 expression comparisons with gastric curvature

Study	Location			P value
	Lesser	Greater	Circumferential	
Matsusaka *etal*[45]	121/550(22.1%)	48/202(23.8%)	60/332(18.1%)	0.395
Indian studies[53-55]	*	*	*	*
Current study	3/21(14.2%)	0/5(0%)	5/46(10.9%)	0.86

* Not evaluated in the particular study

HER 2 AND HISTOLOGICAL CLASSIFICATIONS

A significant correlation between Lauren's intestinal sub type and HER2 positivity has reported in most global and some Indian studies (see Table.17 and18). For this reason, it recommended that, when dealing with a mixed adenocarcinoma (WHO 2010), areas showing an intestinal morphology should selected for HER2 scoring. Our data reinforce the fact that HER 2 positivity mixed adenocarcinoma with tumour heterogeneity is selectively expressed only in differentiated glandular areas. Laurens classified tumours into intestinal and diffuse in 1965. After that many people modified this classification by adding mixed and indeterminate types. We used the original classification with an addition of the general type. We compared HER 2 expression with the latest WHO classification and found a statistically significant expression in mixed adenocarcinoma (p= 0.009%). WHO 2010 classification is a relatively recent classification, and previous authors have not compared HER 2 expression with this classification system. For the detailed comparison between similar studies in the global and Indian population (see table17and 18).

Table 17.HER 2 comparisons with WHO (2010) classification

Study	WHO 2010 classification							P value
	Tubular	pap	PCC	SCC	Mixed	Muc	Others	
Current study	3/25(12%)	0/0(0%)	0/27(0%)	0/6(0%)	5/12(**41.6%**)	0/1(0%)	0/1(0%)	**0.009**
Grabsch etal[58]	13/163(7.9%)	7/38(18.4%)	0/112(0%)		*	2/34(5.9%)	2/7(**28.5%**)	**0.017**

* Not evaluated in the particular study PCC –Poorly cohesive carcinoma, SCC -Signet ring cell carcinoma, PAP- papillary

Table 18.HER 2 comparisons with Laurens classification

Study	Laurens classification			P value
	Intestinal	Diffuse	Mixed	
GERCOR[57]	23/107(**21.5%**)	3/56(5.3%)	6/55(10.9%)	**0.002**
Shan *etal*[44]	109/650(**16.8%**)	13/564(2.3%)	21/249(8.4%)	**0.001**
Fan e*tal*[56]	84/568(**8.4%**)	7/389(1.8%)	*	**0.001**
Laboissiere *etal*[49]	11/61(**18%**)	0/21(0%)	2/33(6.1%)	**0.048**
Hadi *etal*[50]	7/54(13%)	1/11(9.1%)	4/20(25%)	0.567
Current study	4/26(15.4%)	4/44(9.1%)	*	0.572
Adithi *etal*[54]	15/43(**34.8%**)	1/15(6.7%)	*	**0.045**
Rajagopal *etal*[53]	16/49(**32.7%**)	0/11(0%)	*	**0.000**
Gupta*etal*[55]	21/57(**36.8%**)	5/47(10.6%)	*	**0.005**
Sekaran*etal*[30]	13/25(52%)	10/27(37%)	*	0.4

* Not evaluated in the particular study

TUMOR GRADE

We found a higher rate of HER2 positivity in moderately and poorly differentiated types of intestinal adenocarcinoma. This finding is similar to those of the GERCOR study and that by Sekaran an et al. in which HER2 positive cases were moderately differentiated type adenocarcinoma in 65 % and 53 % respectively. Well, differentiated tumours in most of the studies had a meager rate of HER2 positivity. For further detailed comparison between similar research in the global and Indian population (seeTable19)

Table 19.HER 2 comparison with Tumor grade

Study	Tumor grade			P value
	GradeI	GradeII	Grade III	
GERCOR *etal*[57]	3/92(3.3%)	21/55(**38.1%**)	8/71(11.3%)	**0.001**
Shan *etal*[44]	4/25(16%)	74/369(**20.1%**)	65/1069(6.1%)	**0.001**
Fan e*tal*[56]	*	57/324(**17.6%**)	34/633(5.4%)	**0.001**
Laboissiere *etal*[49]	4/13(**30.8%**)	7/45(15.6%)	2/66(3%)	**0.04**
Hadi*etal*[50]	0/4(0%)	9/50(18%)	3/31(9.7%)	0.272
Current study	0/0(0%)	5/29(17.2%)	3/42(7.1%)	0.34
Adithi *etal*[54]	*	12/24(50%)	3/13(23%)	0.111
Gupta *etal*[55]	*	21/48(**43.75%**)	0/9(0%)	**0.01**
Rajagopal *etal*[53]	*	16/40(**40%**)	0/11(0%)	**0.01**
Sekaran *etal*[30]	*	10/19(52.6%)	13/33(39.4%)	0.396

* Not evaluated in the particular study

LYMPHOVASCULAR INVASION AND PERINEURAL INVASION

Table 20.HER 2 comparisons with LVI and PI

	LVI			PI		
Study	Present	Absent	P value	Present	Absent	P value
Laboissiere *etal*[49]	13/94(**18.3%**)	0/30(0%)	**0.031**	*	*	*
Garbish *etal*[58]	15/199(7.5%)	9/219(4.1%)	0.133	*	*	*
Current study	6/28(**21.4%**)	2/44(4.5%)	**0.049**	3/44(6.8%)	5/28(17.9%)	0.248
Adithi *etal*[54]	*	*	*	*	*	*
Gupta *etal*[55]	5/30(16.7%)	1/8(12.5%)	0.89	5/27(18.5%)	1/13(7.7%)	0.66

* Not evaluated in the particular study. LVI -Lympho vascular invasion, PI – Perineural invasion.

DEPTH OF INVASION

Table 21.HER 2 comparisons Depth of invasion

Study	Depth of invasion					
	pT1		pT2	pT3	pT4		P value
	PT1a	PT1b			pT4a	PT4b	
Matsusaka[45]	12/32(37.5%)		19/76(25%)	85/299(28.4%)	119/720(16.5%)	55/256(21.5%)	0.5
Shan [44]	16/206(7.8%)		23/147(**15.6%**)	102/1074(9.5%)	2/36(5.6%)		**0.053**
Hadi[50]	0/0(0%)		4/14(28.6%)	5/45(11.1%)	3/26(11.5%)		0.499
Laboissiere[49]	0/17(0%)		3/32(9.3%)	10/69(14.5%)	0/6(0%)		0.21
Current study	0/3(0%)	0/2(0%)	2/7(28.6%)	5/22(22.7%)	1/38(2.6%)	0/0(0%)	0.248
Gupta l[55]	0/4(0%)		1/5(20%)	3/23(13%)	2/8(25%)		0.598

REGIONAL LYMPH NODE METASTASIS

Table 22.HER 2 comparisons in Regional lymph node metastasis

Study	Regional lymph node metastasis					
				N3		P value
	N0	N1	N2	N3a	N3b	
Matsusaka[45]	23/180(12.7%)	33/140(23.6%)	49/227(21.6%)	65/264(24.6%)	26/188(13.8%)	0.70
Shan[44]	39/411(9.5%)	27/235(11.5%)	20/306(6.5%)	57/511(11.2%)		0.074
Laboissiere[49]	2/39(5.1%)	2/46(4.3%)	3/24(12.5%)	1/15(6.7%)		0.453
Hadi[50]	3/21(14.3%)	2/11(18.2%)	4/25(16%)	3/25(12%)		0.724
Current study	1/12(8.3%)	1/14(7.1%)	2/16(12.5%)	4/23(17.4%)	0/7(0%)	0.859
Indian studies[53-55]	*	*	*	*	*	*

* Not evaluated in the particular study

DISTANT METASTASIS

Table 23.HER 2 comparison distant metastasis

Study	Distant metastasis		P value
	M0	M1	
Shan *etal*[44]	140/1429(9.8%)	3/34(8.8%)	0.571
Fan e*tal*[56]	114/935(12.2%)	8/22**(36.3%)**	**0.001**
Laboissiere *etal*[49]	13/12(10.7%)	0/3(0%)	0.548
Current study	8/71(11.3%)	0/1(0%)	1
Indian studies[53-55]	*	*	*

* Not evaluated in the particular study

PRECANCEROUS CONDITION

In our study, among the 8HER 2 positive cases, 1 case showed active H pylori infection and in one case the adjacent mucosa exhibited dysplasia. So in our study there was no significant correlation between H. pylori and HER2 over-expression. After extensive search of the literature, we found only one study on HER expression in which precancerous conditions were evaluated (see Table 24).

Table 24.HER 2expression-comparison with precancerous conditions

Study	Non identified	H.pylori Gastritis	Intestinal metaplasia	Dysplasia	P value
Current study	6/53(11.3%)	1/11(9.1%)	0/5(0%)	1/3(33.3%)	0.443
Gharsalli etal[51]	4/30(13.3%)	2/13(15.4%)	2/18(11.1%)	*	0.567

* Not evaluated in the particular study

MATCHED BIOPSY WITH RESECTION AND TUMOR HETEROGENEITY

There are very few studies comparing HER 2 expression in matched mucosal biopsies and resection specimens. The prevalence of HER 2 positive cases among resections and biopsies in the current study are 9.72% and 8.3% respectively. The concordance rate of IHC in matching biopsy and surgical specimens is described in the Table 25. When IHC considered as the gold standard, the positive predictive value of biopsies in predicting the final HER2 status was 65%. The NPV was 98 %. Among the 8 HER2 positive cases, biopsies identified 6, and there were two false negative cases. Therefore, 2 (25%) patients by biopsy findings could not have taken advantage of target therapy with Trastuzumab. In Table 26, we summarized the IHC results of the four concordant HER2 positives and the four discordant cases, and we evaluated the homogeneity of IHC staining. The false negative cases can be explained by the heterogeneous expression of the receptor and by the small number and size of biopsy specimens. In our study, out of the 8 HER 2 3+ positive cases, 3 cases received neoadjuvant chemotherapy. 3 cases were HER 2 negative in resections and HER 2 positive in the biopsy. We found that the HER 2 scores are modified by NACT on surgical specimens. Because of this reason, surgical specimens after NACT may-not be reliable for HER2 analysis, possibly because of the absence of residual tumour cells, technical failure and negative shifts. Previous literature has shown that HER2 positive tumour cells have been proven to have higher chemo sensitivity than HER2 negative cells, leading to the difficulty of HER2 detection in

tumours with high pathological responses after NACT(59). All our concordant cases showed a homogeneous pattern of IHC staining, both in surgical and biopsy specimens. The discordance between the HER 2 positivity between resection blocks and biopsies can explain by pre analytical errors, the effect of neoadjuvant chemotherapy and Tumor heterogeneity (see Table 26). Recent studies by GERCOR et al. and PIRRELLI et al. on matched resections and mucosal biopsies to compare HER2 expression scores found a concordance rate similar to our study, mostly due to intratumoral HER2 heterogeneity. Protein expression heterogeneity by IHC usually referred to as variability in immunohistochemical intensity and extension of HER2-positive areas. Intratumoral HER2 heterogeneity significantly impacts sample selection for HER2 testing in gastric carcinoma. Many authors have demonstratedHER2 expression heterogeneity. Whole tissue sections obtained from resected primary gastric tumours adequately represent multiple different subclonal cancer population cells which will avoid false negative staining by HER 2, especially important in mixed adenocarcinoma variant that shows diverse clones of cancer cell populations. For Diagnostic endoscopic biopsies, a recommendation is an adequate, viable number of representative tumour fragments (ideally 6–8) and, consideration needs to given to performing HER2 testing on all available specimens when a negative result found on an endoscopic tumour biopsy(60). Most studies show a higher HER 2 positivity in biopsies compared to surgical specimens (see Table 27). This higher rate could be because of better fixation of tissue and minimal cold ischemic time in small biopsies when compared with resection specimen. But there is some limitation to this hypothesis since most studies are not using the same patient's

biopsy and resection for the comparison. When we compared matched biopsy and resection specimens we got a HER 2 positivity that was similar in biopsy and resection specimens: 8.33% and 9.72% respectively. Analogous to the results of the GERCOR study (see Table 25 and 27). Literature also says that HER 2 positivity is more common in GEJ tumours when compared to other gastric carcinoma (see Table 27). The facts that our study was limited to gastric carcinoma tumours alone and most of the tumours were a poorly differentiated tumour and located in the antrum could explain our low prevalence of 11.1%.

Table 25.HER2 Positivity Concordance rate, PPV, NPV

Study	Biopsy	Excision	Concordance rate	PPV	NPV
GERCOR[57]	14.7%	13.3%	94%	*	*
Pirelli etal[61]	11.5%	*	98%	71.4%	94.4%
Grillo etal[62]	*	*	80%	78.6%	80%
Lee etal[63]	31.2%	8.8	74.1%	*	*
Wang etal[64]	10.1%	11.17	96.1%	*	*
Current study	9.72%	8.3%	95.833	62.5%	98%

* Not evaluated in the particular study

Table 26.HER2 Matched biopsy and resection Discordance and concordance

Cases scenarios	HER2 positive biopsy	HER2 positive Resection	DISCUSSION
Case: (15, 35 ,59,60)	3+	3+	1. Homogeneous staining
Case 62 Case 68	3+ 3+	0 0	1. Pre analytical errors in staining. 2. Effect of neoadjuvant chemotherapy. 3. Tumour heterogeneity.
Case 5 Case 64	0 0	3+ 3+	1. Pre- analytical errors in staining. 2. Tumour heterogeneity 3. Inadequate sampling

Table 27.HER 2 Comparison between biopsy with resection and gastric carcinoma with GEJC

Study	Biopsy	Resection	Study	GEJC	GC
	Matched pairs				
GERCOR[57]	13.3%	14.7%	Gravalo s etal[29]	25%	9.5%
Current study	8.3%	9.72%	Tanneretal[65]	24%	12%
Wang etal[64]	10.1%	11.17%	Shanetal[44]	32%	18%
	Non matched pairs		Lordicketal[66]	32%	18%
			Rajagopaletal[53]	45.5%	22.2%
Lee etal[63]	31.2%	8.8%	Current study	*	11.1%
Aditietal[54]	34.1%	11.8%			

*Not evaluated, GC –Gastric carcinoma, GEJC – Gastro esophageal junction carcinoma.

14/72patients received Neoadjuvant chemotherapy (NAC) and in that 3/14 cases showed HER 2 positivity. In the non-NAC study group 4/5 (80%) HER 2 positive cases showed concordance between biopsy and resection. 1/5 (20%) cases showed discordance with a positive shift (resection HER 2 positive and biopsy negative). In the NAC study group, 3/3 (100%) HER 2 positive cases showed discordance with 2/3(75 %) showing a negative shift (resection HER 2 negative and biopsy positive) and 1/3 (25%) a positive shift. 3/5 (60%) of treated patients showed tumour heterogeneity with a mixed histological type, and the remaining 2(40%) cases showed homogeneous staining pattern in tubular histological type. Our results match with those of the GERCOR study which evaluated two independent cohorts of NAC and none- NAC patients. For detailed analysis and comparison with our study group see (figure 34).

Some authors have also reported extensive cytoplasmic background staining of the gastric foveolar layer and foci of intestinal metaplasia/dysplasia[49], as seen in our study, which suggests an intrinsic clonal characteristic rather than a methodological problem . In fact, HER2 staining of dysplastic epithelium has been correlated to gene amplification by ISH although there were no difficulties in distinguishing HER2-positive neoplastic cells from those areas, we stress that the staining of the latter must not be considered when scoring HER2 expression.

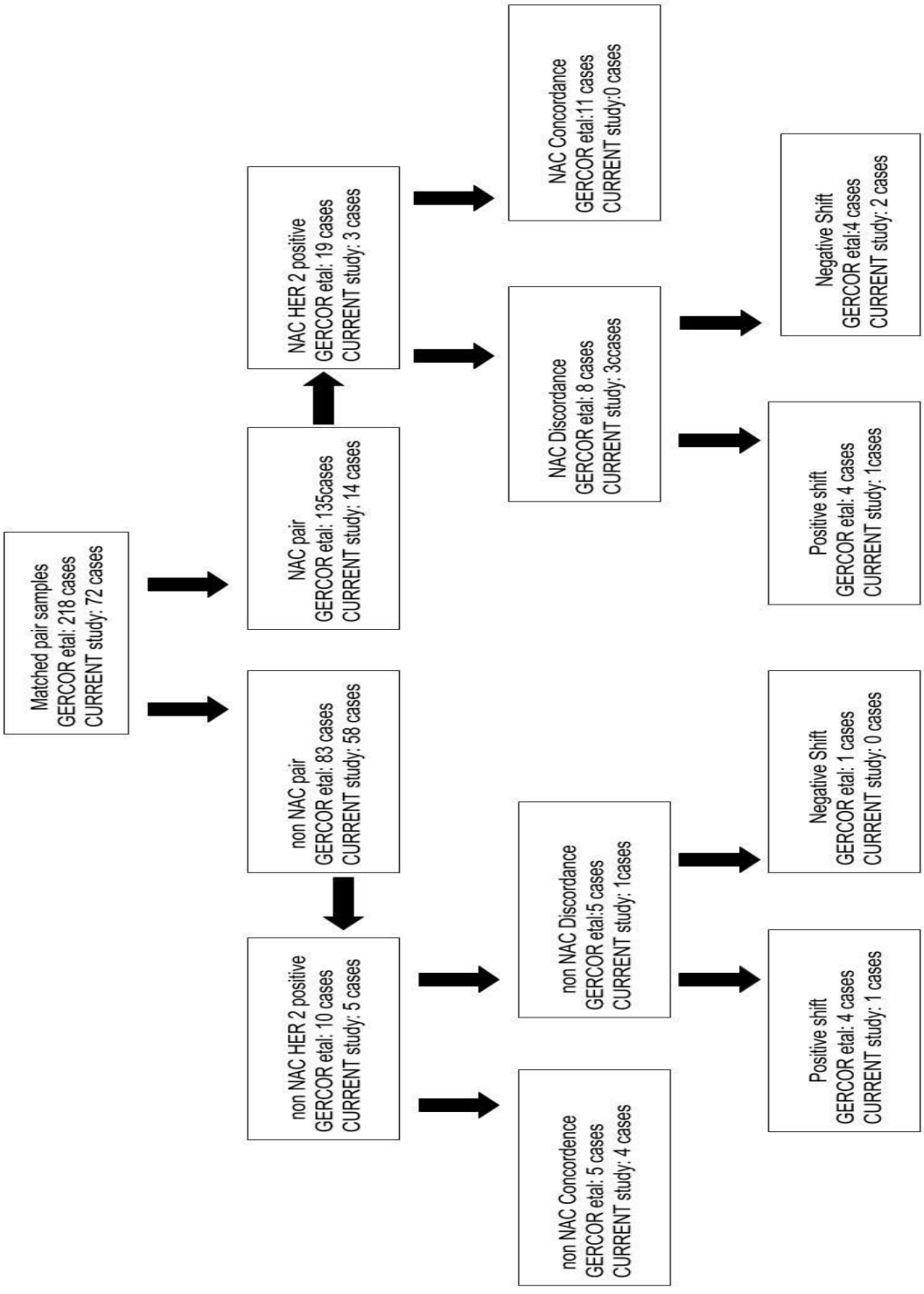

Figure34.Comparison between GERCOR study and Current study between matched biopsy and resection cases and the proportions of the concordance and discordance cases * NAC-Neoadjuvant chemotherapy.

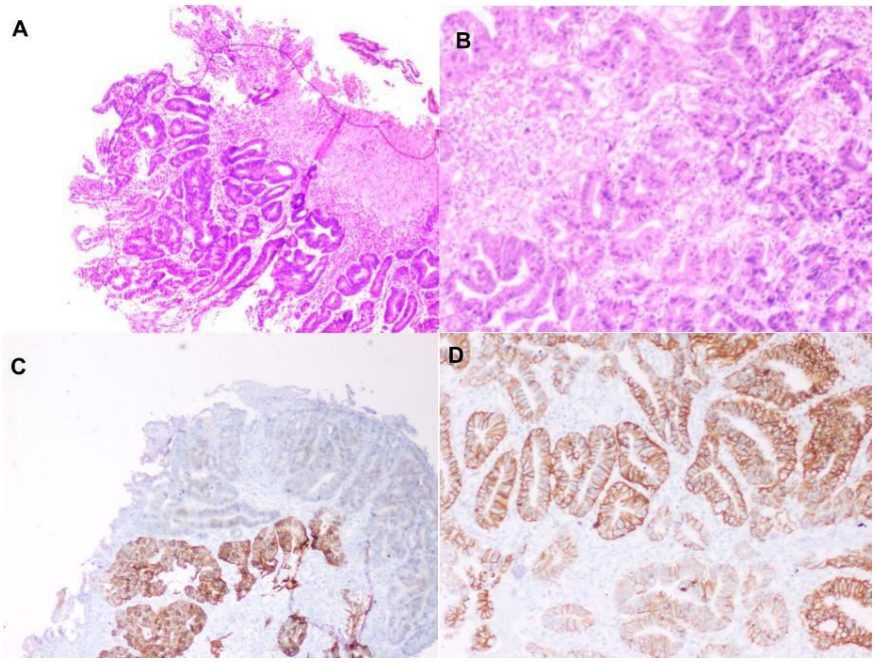

Figure 35. Scenario1. Both biopsy and resection show HER 2(3+) and heterogeneity staining in biopsy as well as in resection with histo morphologically similar area (Case35)

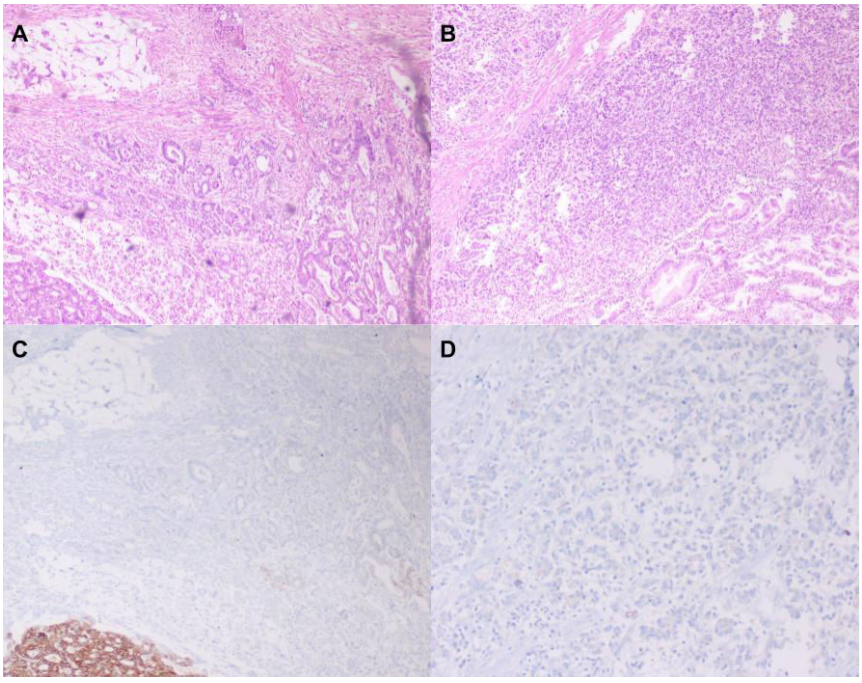

Figure 36.Scenario 2 Resection show 3+ and heterogeneity staining in mixed adeno carcinoma, Selective staining in neoplastic glandular areas. HER 0 in mucinous and poorly cohesive areas (Case 60).

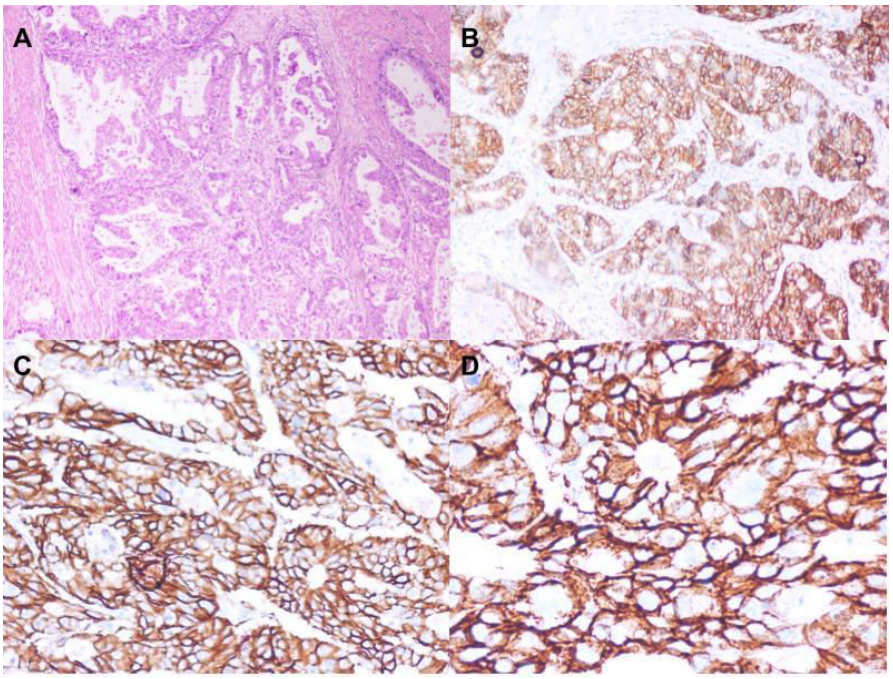

Figure 37.Scenario 3 both biopsy and resection show tubular/intestinal type staining with homogenous staining (Case 15 and 59)

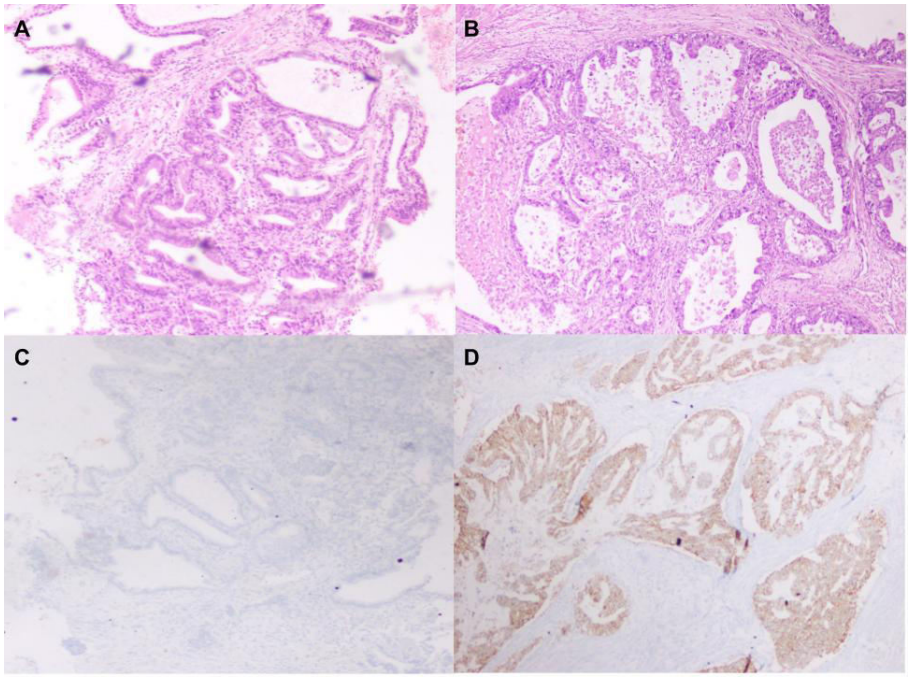

Figure 38.Scenario 4 both biopsy and resection show tubular/intestinal type with Biopsy HER 2 0, and resection HER 3+ (Case 5 and 64)

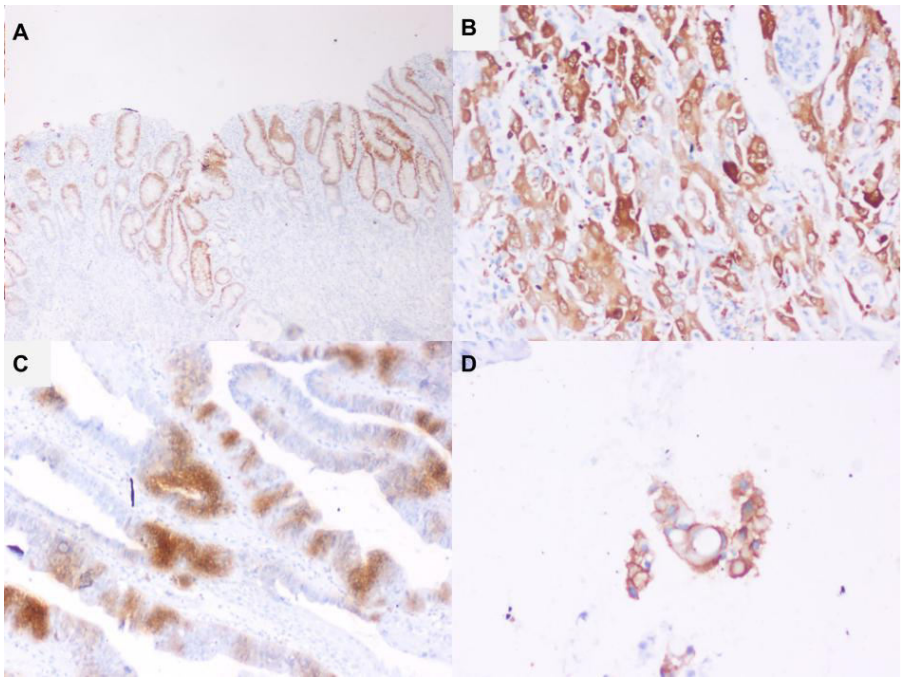

Figure 39.Unusual staining pattern. A-Adjacent normal gastric foveolar epithelium, B- Cytoplasmic and nuclear staining, C-smudgy staining, and D- Very rare scenario staining signet ring cells.

FUTURE DIRECTIONS

HER 2 testing kits have been available in the market from 1996 onwards and have been steadily increased in number over these 15 years. The accuracy and reproducibility of these assays has also improved from time to time. So the next question is whether we should continue to focus our efforts on improving existing HER2 testing methodologies or focus on other potentially better tests for selecting patients who would benefit from HER2-directed therapies(67).Serum tests may simplify the determination of HER2 status testing if a reliable test can develop. Potential candidates for such a test are soluble HER2 (sHER2) extracellular domain (ECD) And HER2 in circulating tumor cells (CTCs). Levels of sHER2 ECD can be accurately quantified in serum using an ELISA, which is

relatively quick and straightforward compared with IHC and FISH. HER2-positive CTCs do not necessarily reflect the HER2 status of a primary tumor but may indicate the status of potential metastatic deposits. HER2 gene amplification and mRNA over expression are intrinsically linked; therefore the utility of quantitative real-time polymerase chain reaction (RT-PCR) has been assessed as a potential alternative to IHC and FISH. The simplicity and accuracy of HER2 testing increased with the current quantitative RT-PCR testing with advantages over current methods such as it is a purely quantitative measurement. Interpretation of data need not need a trained person to interpret. It's also not subject to intraobserver variability and can be standardized, automated and performed on small samples. Another mRNA technique has recently been reported, using automated direct quantification of HER2 mRNA by in situ hybridization. All these advanced techniques have sidesteps problems that can lead to equivocal results in RT-PCR, such as tumor heterogeneity or mixing of a tumor and non-tumor cells during sample preparation. However utilizing this molecular tumor signature may lead to improvements in diagnosis, recurrence prediction, and individualized treatment strategies. The heterogeneity issue has raised questions on whether tumors with< 10% positively stained cells would respond toHER2 targeted therapy, despite classification as HER2 negative with current scoring criteria. In tumors in which strong complete/basolateral or lateral membrane staining seen in< 10% of the cells, IHC staining should be repeated on a different paraffin block section, and if still inconclusive, an ISH test should be performed to determine HER2 gene amplification and final HER2 status(68).

LIMITATION

1. Patient classified as equivocal for HER2 positivity require additional testing (FISH, SISH) to confirm or deny HER2 positivity. Due to cost constraints, this was not planned in our study. Doing FISH would have been helpful for the better understanding of the relation between HER2 heterogeneity and identifying the false positive and negative cases. FISH is the gold standard test for HER 2 amplification assessment.

2. All efforts were taken to minimize the pre analytical errors, but due to logistic reasons, the time taken from the retrieval of gastric tissue to fixing it in formalin was variable and may potentially affect the staining pattern of tumor cells.

3. Most of the patients in our study were from Bangladesh and West Bengal. Our results, therefore, may not reflect the actual prevalence of the condition in the entire Indian population.

4. Our study was a prospective study, so because of the time constraint, follow up of HER 2 positive gastric carcinoma patients were for a limited duration.

5. The number of patients enrolled in this study compared to other international studies is relatively small. Larger numbers are needed to substantiate the findings of this study.

6. We assumed that all resection cases with no glandular differentiation and HER 2 IHC 0 and IHC 1+ would be uniformly HER 2negative. Only HER2 positive 3+ cases we reassessed for HER 2intratumoralheterogeneity. There is a chance that intratumoral heterogeneity may also have been present in other HER2 negative cases.

CONCLUSION

- Our study found HER2 over expressionin11.11% of gastric cancers, similar to most studies in India and the rest of the world.
- HER 2 positivity (3+) was most common in the mixed, intestinal type and moderately differentiated carcinomas.
- We found a statistically significant correlation betweenHER2 over expression and mixed adenocarcinoma (WHO classification) and the presence of lymph vascular invasion in gastric cancers, suggesting that these cases may benefit from targeted therapy using Trastuzumab Diffuse type of gastric cancers not expressing HER2 need to be studied further, to confirm any existing geographic variation.
- In the era of automated techniques and expertise we should pay more attention to prevent pre analytical errors in HER 2 staining by using standard operating protocols in each lab. Importance of the cold ischemic time needs to be further analyzed in gastric resection specimens in future studies.
- Though Trastuzumab is approved for advanced gastric and GEJ cancers, the role of Trastuzumab in adjuvant / neo-adjuvant setting in early stages needs to be evaluated, including the use of newer agents like Pertuzumab and Bevacizumab, especially in young patients.
- Concordance of HER expression between gastrectomies and biopsies in our study was 95.3 %. . The differences between biopsy and resection specimen HER2

expression could be explained by intratumoral heterogeneity and by a decrease in HER2 expression in surgical sections after NAC in patients responding to treatment, possibly due to a higher chemo sensitivity of HER2-positive clones.

- The NPV for HER2 expression in endoscopic biopsies was very high, but the PPV was rather unsatisfactory. This is mainly due to the intrinsic heterogeneity of HER2 expression in gastric carcinoma. A larger number of biopsies and a second set of biopsies to study HER2 expression could be useful in selected inoperable cases, such as those of intestinal type or Mixed type in which a greater probability of achieving a positive result is expected.
- The current study demonstrated significant intratumoral heterogeneity in HER2 protein over expression even without morphological heterogeneity.
- Intratumoral heterogeneity is likely to affect the accuracy of HER2 interpretation by a pathologist and is likely to be the main reason of discordance between endoscopic biopsies versus resection tumor specimens.
- Resection tumor specimens have higher positive rates of HER2 protein over expression, probably because of a larger sample volume.
- The clinical significance of intratumoral heterogeneity and its impact on targeted therapy outcome in gastric cancer requires further studies.

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ANNEXURES

ANNEXURES -1

1.ID no: 2.Age: 3. Sex:

4.Procedure

Partial gastrectomy, proximal partial gastrectomy,distal partial gastrectomy (specify):
Total gastrectomy: Biopsy:

5.Macrocopic findings:

5.1 Anatomical site	Fundus/ cardia	Body	Antrum/ pylorus	Anterior wall	Posterior wall	Lesser curvature	.Greater curvature
Endoscopy							
Radiology							
OR notes							

Other (specify) Not specified

5.2 Greatest dimension: ……mm Additional dimention:
5.3 Macrocopic: AGC: a) polypoidal b) fungating c)ulcerated d)infiltrating

6.Microcopic findings

Lauren's classification(1965)	Modified Lauren's classification	WHO (2010) classification
a)Intestinal	Proximal non diffuse	a1)Papillary a2)Tubular
b)Diffuse	Diffuse	b1)Signet ring b2)Mucinous
c)Indeterminate	Distal non diffuse	c)Uncommon histologic variants

7.Margins:

7.1proximal: 7.2Distal: Omental (radial)

8.Depth of invasion.

8.1) pT0: No evidence of primary tumor 8.2) pTis: Carcinoma in situ/high-grade glandular dysplasia

8.3) pT1a: Tumor invades lamina propria/ muscularis mucosae 8.4) pT1b: Tumor invades submucosa

8.5) pT2: Tumor invades muscularis propria 8.6) pT3: Tumor invades subserosal connective tissue,

without involvement of visceral peritoneum or adjacent structures

8.7) pT4a: Tumor invades serosa (visceral peritoneum) 8.8) pT4b: Tumor invades adjacent structures

9.Regional Lymph Nodes

9.1) pNX: Cannot be assessed 9.2) pN0: No regional L.N metastasis

9.3) pN1: Metastasis in 1 to 2 perigastric L.N 9.4) pN2: Metastasis in 3 to 6 perigastric L.N

9.5) pN3a: Metastasis in 7 to 15 perigastric L.N 9.6) pN3b: Metastasis in 16 or more perigastricL.N

10. Distant Metastasis (pM)

pM1: Distant metastasis Specify site(s), if known: _____

11.Additional Pathologic Findings

11.1 None identif ied 11.2Intestinal metaplasia 11.3) polyp 11.4) Dysplasia Low-grade/High-grade glandular dysplasia. 11.5)gastritis (specify): 11.6 others

12). Histologic Grade

12.1) Grade X Cannot be assessed 12.2) Grade 1 Well differentiated (greater than 95% of tumor composed of glands)

12.3) Grade 2 Moderately differentiated (50% to 95% of tumor composed of glands)

12.4) Grade 3 Poorly differentiated (49% or less of tumor composed of glands)

12.5) Signet-ring cell carcinomas are high grade and are classified as grade 3.

12.6) Small cell neuroendocrine carcinomas and undifferentiated carcinomas are classified as grade 4.

13 Lymphovascular invasion: **14 perineural invasion:**

15 Treatment effect: 15.1Grade0 15.1 Grade1 15.1Grade2 15.1Grade3 15.1Not known

16) IHC:HER 2

16.1) 3+ 16.2) 2+ 16.3)1+ 16.4) 0

ANNEXURES -2

Informed Consent form to participate in a research study

Study Title: CORRELATION OF THE IMMUNOHISTOCHEMICAL EXPRESSION OF HER2 WITH TUMOUR MORPHOLOGY AND TNM STAGE IN MUCOSAL BIOPSIES AND CORRESPONDING TOTAL OR SUBTOTAL GASTRECTOMY SPECIMENS OF PATIENTS WITH ADENOCARCINOMA

Study Number:

Subject's Initials:

Subject's Name:

Date of Birth / Age:

(Subject)

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable

Date: _____/_____/_____

Signatory's Name: Signature:

Or Representative:

_____

Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness:

_____

Date: _____/_____/_____

Name & Address of the Witness: _____

CORRELATION OF THE IMMUNOHISTOCHEMICAL EXPRESSION OF HER2 WITH TUMOUR MORPHOLOGY AND TNM STAGE IN MUCOSAL BIOPSIES AND CORRESPONDING TOTAL OR SUBTOTAL GASTRECTOMY SPECIMENS OF PATIENTS WITH ADENOCARCINOMA

Patient Information Sheet

Gastric cancer is one of the most common malignancies worldwide.Survival of such patients can be poor because of its non specific presentation and late diagnosis. The gastric cancers can be in different locations within the stomach and also be of different types. In this study we are looking for what are all the different types of gastric cancer and also if there is a particular gene within the gastric cancers called the “HER2 “. This type of cancer may be a more serious variety and may require different treatment.

You/Your patient have been found to have probable gastric carcinoma on endoscopy/Imaging. Your doctor has referred you for undergoing endoscopies and biopsies. This biopsy is absolutely needed for proper diagnosis and further treatment in your case. Along with the above test nothing extra will be taken for our study during the above endoscopy procedure. You will not be subjected to any other additional procedure and there is no additional cost for this study. The tissue taken during your routine biopsy will also be tested for HER2 expression as well. The information obtained may help improve the care for you and other patients in future without subjecting you to any additional risk.

Participating in this study does not expose you to additional risk other than the risk usually associated with endoscopy and biopsies, which are discomfort in your throat during the procedure and very

rarely, chance of bleeding or perforation (about 3 patients per 10000 patients biopsied). However we will provide for care in case 0f any complications related to the endoscopy and biopsy happens to you.

Your personal information will be kept confidential. The data gathered will be analyzed by a single person and data reported will be for the group, without identification of individual patients. We will also provide you with the contact details of the person you may get in touch with in case of queries. The participation in this study is voluntary and you can withdraw at any point of time. Withdrawal from the study will not affect your treatment prospects in our hospital.

Contact details:

[redacted]

[redacted]

General pathology
Asha building 4 th floor
CMC Vellore

[redacted]

ANNEXURES -4

IRB approval letter.

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

February 11, 2017

[redacted]
PG Registrar,
Department of Pathology,
Christian Medical College,
Vellore 632 004.

Sub: **Fluid Research Funding: New Proposal**
Correlation of the immune histochemical expression of her2with tumour morphology and tnm stage inmucosal biopsies and corresponding total or subtotal gastrectomy specimens of patients with adenocarcinoma
[redacted] Pulimood, Employment Number:14466, General Pathology, Dr. Dipti Masih, General Pathology, Dr.Inian.S, General surgery, Dr Raju Titus Chacko, medical oncology, Dr Reuben Thomas, G.I.Sciences.

Ref: IRB Min No: 10206 [OBSERVE] dated 08.08.2016

[redacted]
The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Correlation of the immune histochemical expression of her2with tumour morphology and tnm stage inmucosal biopsies and corresponding total or subtotal gastrectomy specimens of patients with adenocarcinoma" on August 08th 2016.

The Committee reviewed the following documents:

1. IRB Application format
2. Cvs of Drs. Anna Pulimood, Inian Samarasam, Anand KV, Dipti, Raju Titus, C, Ruben and Prasanna.
3. Informed Consent forms of Participation
4. Patient information sheet
5. Proforma
6. No. of documents 1 – 5

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on August 08th 2016 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002. 2 of 4

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC,Vellore	Internal, Clinician
Dr. B. J. Prashantham	MA(Counseling Psychology), MA (Theology), Dr. Min (Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. Anuradha Rose	MBBS, MD, MHSC (Bioethics)	Associate Professor, Community Health, CMC, Vellore	Internal, Clinician
Dr. Jayaprakash Muliyil	BSc, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, Vellore	External, Scientist &Epidemiologist
Rev. Joseph Devaraj	BSc, BD	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Visalakshi. J	MPH, PhD	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mrs. Sheela Durai	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Ms. Grace Rebekha	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person
Dr. Rajesh Kannangai	MD, PhD.	Professor, Clinical Virology, CMC, Vellore	Internal, Clinician
Dr. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294. 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. Sneha Varkki	MBBS, DCH, DNB	Professor, Paediatrics, CMC, Vellore	Internal, Clinician
Mrs. Emily Daniel	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Dr. Sathish	MBBS, MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician
Dr. Inian Samarasam	MS, FRCS, FRACS	Professor, Surgery, CMC, Vellore	Internal, Clinician
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC, Vellore	Internal, Clinician
Dr. Ranjith K Moorthy	MBBS, MCh	Professor, Neurological Sciences, CMC, Vellore	Internal, Clinician

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "Correlation of the immune histochemical expression of her2with tumour morphology and tnm stage inmucosal biopsies and corresponding total or subtotal gastrectomy specimens of patients with adenocarcinoma" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:

A sum of 50,000/- INR (Rupees Fifty thousand Only) will be granted for 12 months.

Yours sincerely,

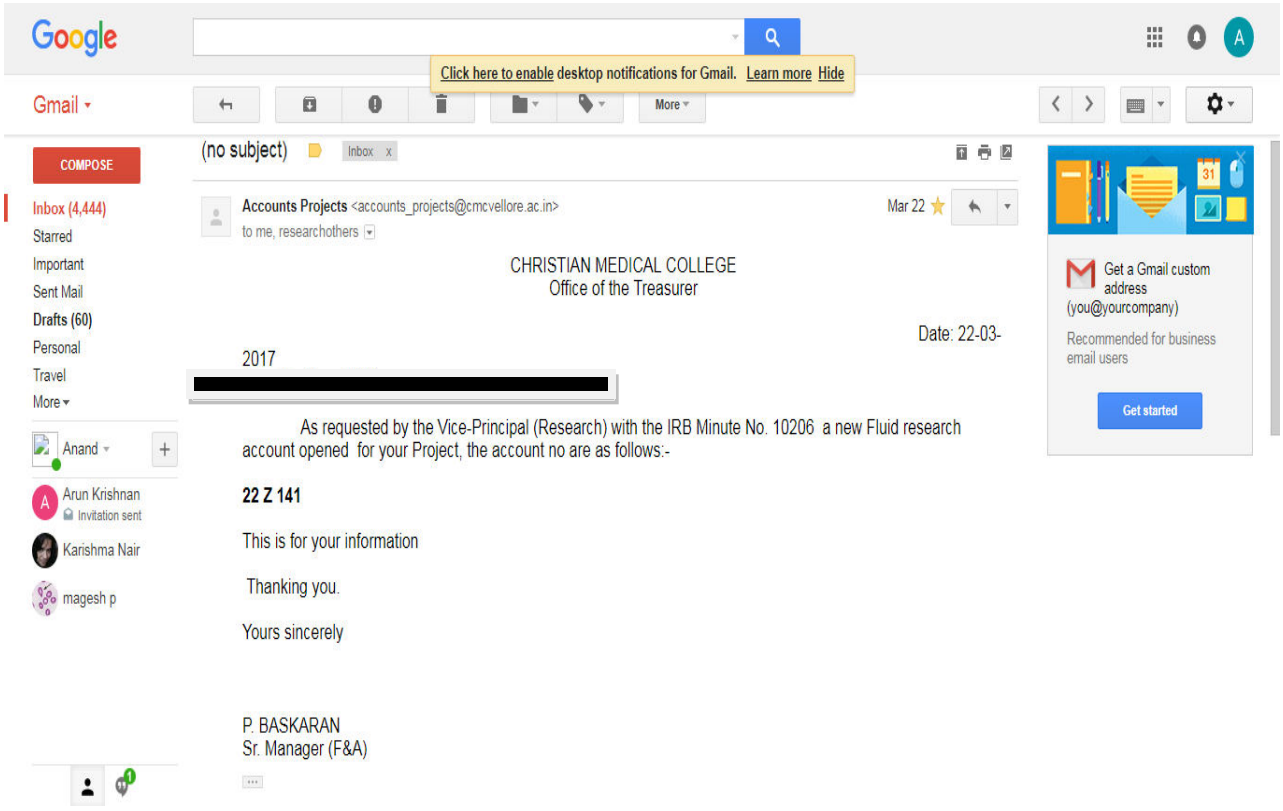
Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS, MD, DM,
SECRETARY (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min No: 10206 [OBSERVE] dated 08.08.2016

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294. 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

ANNEXURES -5



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Accounts Projects <accounts_projects@cmcvellore.ac.in> Mar 22
to me, researchothers

CHRISTIAN MEDICAL COLLEGE
Office of the Treasurer

Date: 22-03-2017

[redacted]

As requested by the Vice-Principal (Research) with the IRB Minute No. 10206 a new Fluid research account opened for your Project, the account no are as follows:-

22 Z 141

This is for your information

Thanking you.

Yours sincerely

P. BASKARAN
Sr. Manager (F&A)

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Get started

Slandered operating protocol for HER 2 IHC staining

Details of the HER 2 IHC staining is as follows:

Ventana (Pathway) anti HER 2/neu (4B5) Rabbit Monoclonal Primary Antibody procedure - MILD- 32 CC1 PROTOCOL

PATHWAY HER2 (4B5) is a rabbit monoclonal antibody, which binds to HER2 in paraffin embedded tissue sections. The specific antibody can be localized by either a biotin conjugated secondary antibody formulation that recognizes rabbit immunoglobulins followed by the addition of a streptavidin-horseradish peroxides (HRP) conjugate a secondary antibody-HRP conjugate (Ultra view Universal DAB detection kit). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. Each step is incubated at a precise time and temperature. At the end of each incubation step, the Ventana automated slide stainer washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies Liquid Coverslip™, which minimizes evaporation of the aqueous reagents from thespecimen slide. Clinical cases should be evaluated within the context of the performance of appropriate controls. Ventana recommends the inclusion of a positive tissue control fixedand processed in the same manner as the patient specimen (for example, positive breast carcinoma or uterus).

ANNEXURES -7

Important scoring system

Staining	Surgical specimen	Biopsy	Interpretation
0	No reactivity or membranous reactivity in <10% of tumour cells	No reactivity or no membranous reactivity in any tumour cell	Negative
1+	Faint or barely perceptible membranous reactivity in ≥10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster with a faint or barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Equivocal
3+	Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Positive

WHO 2010 Gastric adenocarcinoma classification

8260 Papillary adenocarcinoma: composed of well-differentiated Exophytic carcinoma with elongated finger-like processes lined by cylindrical or cuboidal cells supported by fibrovascular connective tissue cores. The cells tend to maintain their polarity. Some tumours show tubular (papilla tubular) differentiation. Rarely, micropapillary architecture is present. The degree of cellular atypia and mitotic index vary; there may be severe nuclear atypia. The invading edge of the tumour is usually sharply demarcated; the tumour may be infiltrated by acute and chronic inflammatory cells.

8211 Tubular adenocarcinoma: composed of the predominance of tube-like epithelial structures. Tubular adenocarcinoma of the stomach is composed of dilated or slit-like and branching tubules of varying diameter. Acinar structures may also be present

8480 Mucinous adenocarcinoma: composed of malignant epithelium and extracellular mucinous pools. By convention, the tumour shows more than 50% extracellular mucin. Mucinous carcinomas may contain scattered signet-ring cells.

8490 Signet ring cell carcinoma: Signet ring cell carcinoma is predominantly composed of signet-ring cells containing aclear droplet of cytoplasmic mucin displacing the nucleus.

8490 Poorly cohesive carcinoma:Tumor cells infiltrate as isolated single cells or small aggregates. Signet ringastric carcinoma ell carcinoma is predominantly composed of

signet-ring cells containing aclear droplet of cytoplasmic mucin displacing the nucleus. Other variants ofpoorly cohesive carcinoma may resemble mononuclear inflammatory cells

8255: Mixed adenocarcinoma: Mixed carcinomas display a mixture of discrete morphologically identifiable glandular (tubular/papillary) and signet-ring/poorly-cohesive cellular histological components. Any discrete histological component should be reported; although the prognostic relevance of the proportion of each component has not been established, preliminary data suggest that any signet-ring/poorly cohesive cellular histological component is associated with a poor prognosis.Mixed carcinomas are clonal and phenotypic divergence has been attributed to the somatic mutation in the E-cadherin gene (CDH1), which is restricted to the signet-ring/poorly-cohesive component.

8560 Adenosquamous carcinoma: Mixture of glandular and squamous neoplastic components; the squamous

the component should comprise at least 25% of tumour volume.

8512 Carcinoma with lymphoid stroma: This tumour, also reported as lymphoepithelioma-like carcinoma or medullary carcinoma, is characterized by poorly developed tubular structures associated with a prominent lymphoid infiltration of the

stroma. These tumours frequently affect the proximal stomach or gastric stump and are more common in males while > 80% are associated with infection with Epstein-Barr virus (EBV) 1. The role of EBV in carcinogenesis is debated but occurs at an early stage since EBV can be found in adjacent dysplasia. The prognosis for patients with these tumours is reportedly better than that for patients with typical gastric cancers.

8214 Parietal cell carcinoma: composed of well to moderately differentiated tubular adenocarcinoma with very eosinophilic, finely granular cytoplasm. Immunohistochemical stains for anti mitochondrial antibody were strongly positive.

8576 Hepatoid adenocarcinoma: Hepatoid adenocarcinoma of the stomach is composed of large polygonal eosinophilic hepatocyte-like neoplastic cells. α-Fetoprotein (AFP) can be detected in situ, but also in the serum. Bile and periodic acid-Schiff (PAS)- positive and diastase-resistant intracytoplasmiceosinophilic globules can be observed 3. Other rare AFP-producing carcinomas include well-differentiated papillary or tubular-type adenocarcinoma with clear cytoplasm and yolk-sac tumour-like carcinoma

8070 Squamous cell carcinoma, NOS: A carcinoma arising from squamous epithelial cells, morphologically characterized by the proliferation of atypical, often pleomorphic squamous cells. Squamous cell carcinomas are graded as well, moderately, or poorly differentiated. Well, differentiated carcinomas are usually associated with keratin

production and the presence of intercellular bridges between adjacent cells. Squamous cell carcinoma of the stomach is very rare

8082 Lymphoepithelial carcinoma: Lymphoepithelioma-like carcinoma (LLC) is a rare and peculiar type of gastric carcinoma that is reported to be associated with latent Epstein-Barr virus (EBV) infection. Histopathologically, the carcinoma cell nests were surrounded by prominent lymphoid stroma. Sarcoid-like epithelioid granulomas were noted both in the tumourstroma and in the regional lymph node with metastasis.

8510 Medullary carcinoma, NOS:

8020 Undifferentiated carcinoma: High-grade carcinoma that cannot be further classified as adenocarcinoma,

squamous cell carcinoma, or other recognized variants

8246 Neuroendocrine carcinoma: A neuroendocrine carcinoma (NEC) is a poorly differentiated, high grade malignant neoplasm composed of small cells or large to intermediate cells, sometimes with organoid features resembling NET, diffusely expressing the general markers of neuroendocrine differentiation (diffuse expression of synaptophysin; faint or focal staining for chromogranin A), with marked nuclear atypia, multifocal necrosis and a high number of mitoses (> 20 per 10 HPF); high grade (G3) defined according to the proliferation fraction and histology. This definition refers to neoplasms previously classified as small cell carcinoma, large cell (neuro)endocrine carcinoma, or poorly differentiated (neuro)endocrine carcinoma

8013 Large cell neuroendocrine carcinoma: A usually aggressive carcinoma composed of large malignant cells which display neuroendocrine characteristics. It is characterized by the presence of high mitotic activity and necrotic changes.

8041 Small cell neuroendocrine carcinoma: A neuroendocrine carcinoma composed of small malignant cells which histologically often resemble "oat cells". Clinically, this is often a rapidly growing cancer that spreads to distant sites early.

8244 Mixed adenoneuroendocrine carcinoma: Mixed adenoneuroendocrine carcinomas (MANEC) have a phenotype that is morphologically recognizable as both gland-forming epithelial and neuroendocrine, and are defined as carcinomas since both components are malignant and should be graded. A component of squamous cell carcinoma is rare. Arbitrarily, at least 30% of either component should be identified to qualify for this definition. The identification in adenocarcinoma of scattered neuroendocrine cells by immune histochemistry does not qualify for this definition

ANNEXURES -9

Macroscopic - Bormann classification

The Type 1 Polypoid: Well circumscribed polypoidtumours. Polypoid carcinoma of the stomach is located in the antrum of the lesser curvature. This elevating solid mass shows focal superficial haemorrhage.

Type 2 Fungating: Fungatingtumours with marked central infiltration and most of the time with a central ulceration. The most common type. Lesser curvature of the antrum is the most common site.

Type 3 Ulcerated: Ulcerated tumours with infiltrative margins. Ulcerated carcinoma of the stomach with infiltrative and heaped-up margins is present The lesser curvature near the body Polypoid (type 1), and ulcerated (type 3) types are commonly found in the greater curvature.

Type 4 Infiltrating: Diffusely infiltrated tumours. Linitis plastic, diffusely infiltrating carcinoma of the stomach with thickening of gastric rugae involves the whole stomach

ANNEXURE-10

Table 28. Tumor Regression Grade

	Tumor Regression Grade	
0	No viable cancer cells 0	(Complete response)
1	Single cells or small groups of cancer cells	(Moderate response)
2	Residual cancer outgrown by fibrosis.	(Minimal response)
3	extensive residual cancer	(Poor response)

Table 29.HER 2 standard operating methods

Assay type	Trade name	Manufacturer	Date of FDA approval
Semi-quantitative IHC	HercepTest™	DAKO	September 1998
IHC	PATHWAY	Ventana Medical Systems Inc	November 2000
IHC	InSite	Biogenex Laboratories Inc	December 2004
Semi-quantitative IHC	Bond Oracle™	Leica Biosystems	April 2012

WHO common histological type

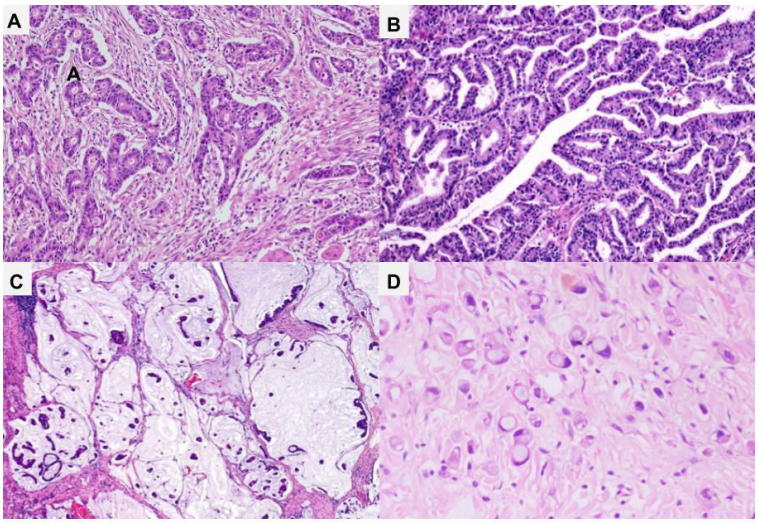

Figure 40.Common WHO subtype A-Tubular, B-papillary, C-Mucinous, D-Signet ring cell

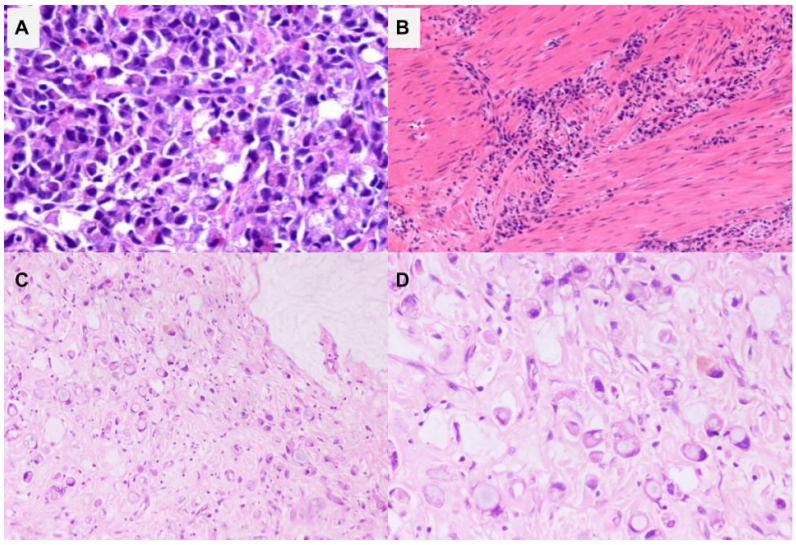

Figure 41.Diffuse type carcinoma subtype. A&B – Poorly cohesive carcinoma. C&D- Signet ring cell carcinoma

ANNEXURES -11

Table 30.DATA SHEETS

NO	AGEBAR	AGE	SEX	PLACE	SURG	LOCATION	CURVE	SIZE	Bormann	LRN	M.LRN	WHO	PM	DM
1	<50 yrs	66	male	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	33	TYPE III	DIFFUSE	D	PCC	NOT INV	NOT INV
2	<50 yrs	62	male	W.B	TOTAL	FUNDUS	CIRCUMF	47	TYPE III	DIFFUSE	D	MIXED	NOT INV	NOT INV
3	>50 yrs	23	female	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	50	TYPE IV	DIFFUSE	D	MIXED	NOT INV	NOT INV
4	<50 yrs	53	female	T.N	D.SUBTOTAL	D.ANTRUM	CIRCUMF	60	TYPE IV	DIFFUSE	D	PCC	NOT INV	INV
5	<50 yrs	55	male	W.B	D.SUBTOTAL	D.ANTRUM	LESSER	20	TYPE III	INTESTINAL	DND	MIXED	NOT INV	NOT INV
6	>50 yrs	32	female	T.N	TOTAL	D.ANTRUM	CIRCUMF	120	TYPE IV	DIFFUSE	D	SCC	INV	INV
7	<50 yrs	54	male	B.DESH	TOTAL	FUNDUS	LESSER	70	TYPE III	INTESTINAL	PND	TUBULAR	NOT INV	NOT INV
8	<50 yrs	55	female	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	50	TYPE IV	DIFFUSE	D	PCC	NOT INV	NOT INV
9	<50 yrs	68	male	W.B	TOTAL	BODY	LESSER	80	TYPE II	INTESTINAL	PND	TUBULAR	NOT INV	NOT INV
10	<50 yrs	73	male	J.KHAND	TOTAL	D.ANTRUM	LESSER	60	TYPE II	DIFFUSE	D	PCC	NOT INV	NOT INV
11	>50 yrs	35	female	B.DESH	TOTAL	D.ANTRUM	CIRCUMF	10	TYPE II	DIFFUSE	D	MIXED	NOT INV	NOT INV
12	<50 yrs	51	male	B.DESH	D.SUBTOTAL	D.ANTRUM	LESSER	65	TYPE II	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
13	>50 yrs	43	male	T.N	D.SUBTOTAL	D.ANTRUM	LESSER	30	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
14	<50 yrs	61	female	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	40	TYPE IV	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
15	<50 yrs	57	male	B.DESH	TOTAL	FUNDUS	LESSER	80	TYPE II	INTESTINAL	PND	TUBULAR	NOT INV	NOT INV
16	>50 yrs	41	male	B.DESH	D.SUBTOTAL	BODY	LESSER	110	TYPE II	DIFFUSE	D	PCC	NOT INV	NOT INV
17	<50 yrs	58	male	T.N	D.SUBTOTAL	D.ANTRUM	GREATER	70	TYPE II	DIFFUSE	D	PCC	NOT INV	NOT INV
18	>50 yrs	41	female	J.KHAND	D.SUBTOTAL	D.ANTRUM	CIRCUMF	80	TYPE IV	DIFFUSE	D	SCC	NOT INV	NOT INV
19	>50 yrs	45	male	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	80	TYPE II	INDETER	D	MUC	NOT INV	NOT INV
20	<50 yrs	59	female	B.DESH	D.SUBTOTAL	D.ANTRUM	CIRCUMF	50	TYPE II	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
21	<50 yrs	57	male	J.KHAND	D.SUBTOTAL	BODY	CIRCUMF	80	TYPE II	DIFFUSE	D	PCC	NOT INV	NOT INV
22	<50 yrs	60	male	T.N	D.SUBTOTAL	D.ANTRUM	CIRCUMF	50	TYPE III	DIFFUSE	D	MIXED	NOT INV	NOT INV
23	<50 yrs	65	male	W.B	D.SUBTOTAL	D.ANTRUM	LESSER	15	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
24	<50 yrs	62	male	T.N	D.SUBTOTAL	D.ANTRUM	LESSER	30	TYPE II	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
25	>50 yrs	37	male	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	90	TYPE IV	DIFFUSE	D	SCC	NOT INV	NOT INV
26	>50 yrs	46	male	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	65	TYPE II	DIFFUSE	D	PCC	NOT INV	NOT INV
27	<50 yrs	54	male	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	25	TYPE III	DIFFUSE	D	PCC	NOT INV	NOT INV
28	<50 yrs	53	male	B.DESH	D.SUBTOTAL	D.ANTRUM	LESSER	70	TYPE IV	DIFFUSE	D	SCC	NOT INV	NOT INV
29	>50 yrs	29	female	B.DESH	D.SUBTOTAL	D.ANTRUM	GREATER	25	TYPE II	DIFFUSE	D	PCC	NOT INV	INV
30	>50 yrs	36	female	J.KHAND	D.SUBTOTAL	D.ANTRUM	CIRCUMF	40	TYPE II	DIFFUSE	D	PCC	NOT INV	NOT INV
31	<50 yrs	69	female	B.DESH	D.SUBTOTAL	D.ANTRUM	CIRCUMF	60	TYPE IV	DIFFUSE	D	PCC	NOT INV	INV
32	<50 yrs	68	male	T.N	D.SUBTOTAL	D.ANTRUM	CIRCUMF	40	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
33	>50 yrs	35	male	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	15	TYPE III	DIFFUSE	D	PCC	NOT INV	NOT INV
34	<50 yrs	64	male	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	45	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
35	>50 yrs	32	male	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	70	TYPE III	DIFFUSE	D	MIXED	NOT INV	NOT INV
36	<50 yrs	51	female	B.DESH	D.SUBTOTAL	D.ANTRUM	CIRCUMF	40	TYPE IV	DIFFUSE	D	PCC	NOT INV	NOT INV
37	<50 yrs	79	male	B.DESH	D.SUBTOTAL	D.ANTRUM	CIRCUMF	35	TYPE IV	DIFFUSE	D	PCC	NOT INV	NOT INV

38	<50 yrs	60	female	J.KHAND	TOTAL	D.ANTRUM	CIRCUMF	15	TYPE IV	DIFFUSE	D	PCC	NOT INV	NOT INV
39	<50 yrs	72	female	T.N	D.SUBTOTAL	D.ANTRUM	CIRCUMF	90	TYPE II	DIFFUSE	D	PCC	NOT INV	NOT INV
40	<50 yrs	67	male	B.DESH	D.SUBTOTAL	D.ANTRUM	LESSER	60	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
41	<50 yrs	61	female	B.DESH	TOTAL	FUNDUS	LESSER	60	TYPE IV	DIFFUSE	D	PCC	NOT INV	NOT INV
42	<50 yrs	57	male	NEPAL	D.SUBTOTAL	D.ANTRUM	LESSER	45	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
43	<50 yrs	60	male	B.DESH	D.SUBTOTAL	D.ANTRUM	GREATER	11	TYPE III	INTESTINAL	PND	TUBULAR	NOT INV	NOT INV
44	<50 yrs	51	male	T.N	D.SUBTOTAL	D.ANTRUM	CIRCUMF	45	TYPE II	DIFFUSE	D	MIXED	NOT INV	NOT INV
45	>50 yrs	42	male	T.N	TOTAL	D.ANTRUM	LESSER	80	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
46	>50 yrs	48	female	B.DESH	TOTAL	WHOLE	CIRCUMF	20	TYPE IV	DIFFUSE	D	SCC	NOT INV	NOT INV
47	<50 yrs	54	female	ODISSA	D.SUBTOTAL	D.ANTRUM	CIRCUMF	10	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
48	<50 yrs	58	female	T.N	TOTAL	FUNDUS	CIRCUMF	100	TYPE IV	DIFFUSE	D	SCC	INV	NOT INV
49	<50 yrs	51	male	B.DESH	TOTAL	WHOLE	CIRCUMF	50	TYPE III	DIFFUSE	D	MIXED	NOT INV	INV
50	<50 yrs	82	male	T.N	D.SUBTOTAL	D.ANTRUM	CIRCUMF	50	TYPE II	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
51	<50 yrs	70	male	W.B	D.SUBTOTAL	D.ANTRUM	GREATER	35	TYPE III	DIFFUSE	D	PCC	NOT INV	NOT INV
52	<50 yrs	70	male	T.N	TOTAL	FUNDUS	LESSER	35	TYPE III	INTESTINAL	PND	TUBULAR	NOT INV	NOT INV
53	>50 yrs	46	male	B.DESH	D.SUBTOTAL	D.ANTRUM	CIRCUMF	12	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
54	<50 yrs	59	male	J.KHAND	D.SUBTOTAL	D.ANTRUM	CIRCUMF	50	TYPE III	DIFFUSE	D	PCC	NOT INV	NOT INV
55	>50 yrs	45	male	W.B	D.SUBTOTAL	D.ANTRUM	LESSER	7	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
56	<50 yrs	59	female	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	30	TYPE III	DIFFUSE	D	PCC	NOT INV	NOT INV
57	>50 yrs	48	male	A.P	D.SUBTOTAL	D.ANTRUM	CIRCUMF	60	TYPE III	DIFFUSE	D	PCC	NOT INV	NOT INV
58	<50 yrs	60	male	J.KHAND	D.SUBTOTAL	D.ANTRUM	LESSER	25	TYPE III	DIFFUSE	D	PCC	NOT INV	NOT INV
59	>50 yrs	35	female	T.N	D.SUBTOTAL	D.ANTRUM	CIRCUMF	70	TYPE II	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
60	<50 yrs	66	male	B.DESH	D.SUBTOTAL	D.ANTRUM	CIRCUMF	30	TYPE IV	DIFFUSE	D	MIXED	NOT INV	NOT INV
61	>50 yrs	49	male	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	25	TYPE III	INDETER	DND	LYMPOID	NOT INV	NOT INV
62	<50 yrs	53	male	W.B	TOTAL	FUNDUS	CIRCUMF	60	TYPE III	DIFFUSE	D	MIXED	INV	INV
63	<50 yrs	56	male	W.B	TOTAL	FUNDUS	LESSER	20	TYPE III	INTESTINAL	PND	TUBULAR	NOT INV	NOT INV
64	>50 yrs	24	male	T.N	TOTAL	FUNDUS	LESSER	60	TYPE III	DIFFUSE	D	MIXED	NOT INV	NOT INV
65	>50 yrs	44	male	B.DESH	D.SUBTOTAL	D.ANTRUM	CIRCUMF	60	TYPE IV	DIFFUSE	D	PCC	NOT INV	NOT INV
66	<50 yrs	59	male	W.B	D.SUBTOTAL	D.ANTRUM	LESSER	35	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
67	>50 yrs	39	female	W.B	D.SUBTOTAL	D.ANTRUM	GREATER	50	TYPE II	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
68	<50 yrs	63	male	B.DESH	D.SUBTOTAL	D.ANTRUM	CIRCUMF	20	TYPE III	INTESTINAL	DND	TUBULAR	NOT INV	NOT INV
69	>50 yrs	43	male	W.B	D.SUBTOTAL	D.ANTRUM	CIRCUMF	50	TYPE II	DIFFUSE	D	MIXED	NOT INV	NOT INV
70	>50 yrs	49	female	A.P	D.SUBTOTAL	D.ANTRUM	CIRCUMF	80	TYPE IV	DIFFUSE	D	PCC	NOT INV	NOT INV
71	<50 yrs	51	male	T.N	D.SUBTOTAL	D.ANTRUM	CIRCUMF	60	TYPE III	DIFFUSE	D	PCC	NOT INV	NOT INV
72	>50 yrs	49	female	A.P	D.SUBTOTAL	D.ANTRUM	CIRCUMF	80	TYPE II	DIFFUSE	D	PCC	NOT INV	NOT INV

CONTD……

NO	DEPTH	RLN	MO	STAGE	PRECAN	GRADE	LVI	PI	NACT	TMR	RESP	RSCORE	BSCORE	RSTAT	BSTAT
1	T3	N1	M0	IIB	H.PYLORI	III	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
2	T4a	N2	M0	IIIA	NON	III	PRESENT	PRESENT	YES	2	POOR	0	0	NEGATIVE	NEGATIVE
3	T4a	N3a	M0	IIIB	NON	II	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
4	T4a	N3a	M0	IIIB	H.PYLORI	III	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
5	T2	N0	M0	IB	H.PYLORI	II	ABSENT	ABSENT	NO	.	.	3	3	POSITIVE	POSITIVE
6	T4a	N3a	M0	IIIB	NON	III	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
7	T4a	N1	M0	IIIA	NON	I	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
8	T4a	N2	M0	IIIA	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
9	T4a	N3a	M1	IV	NON	II	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
10	T4a	N3b	M0	IIIC	NON	III	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
11	T4a	N3a	M0	IIIC	NON	II	PRESENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
12	T3	N1	M0	IIB	H.PYLORI	II	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
13	T3	N0	M0	IIA	NON	II	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
14	T4a	N0	M0	IIB	NON	II	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
15	T3	N3a	M0	IIIB	NON	II	PRESENT	PRESENT	NO	.	.	3	3	POSITIVE	POSITIVE
16	T4a	N3b	M0	IIIC	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
17	T4a	N2	M0	IIIA	NON	III	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
18	T4a	N3b	M0	IIIC	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
19	T4a	N3a	M0	IIIB	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
20	T4a	N3a	M0	IIIB	H.PYLORI	II	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
21	T4a	N3a	M0	IIIB	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
22	T3	N3a	M0	IIIB	NON	III	ABSENT	ABSENT	NO	.	.	1	1	NEGATIVE	NEGATIVE
23	T2	N1	M0	IIA	IM	II	ABSENT	ABSENT	NO	.	.	1	1	NEGATIVE	NEGATIVE
24	T4a	N2	M0	IIIA	H.PYLORI	II	ABSENT	PRESENT	NO	.	.	1	1	NEGATIVE	NEGATIVE
25	T4a	N3a	M0	IIIB	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
26	T3	N3a	M0	IIIB	H.PYLORI	III	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
27	T1a	N1	M0	IB	IM	III	PRESENT	ABSENT	YES	1	GOOD	0	0	NEGATIVE	NEGATIVE
28	T4a	N3a	M0	IIIB	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
29	T4a	N3b	M0	IIIC	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
30	T3	N3a	M0	IIIB	NON	III	PRESENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
31	T4a	N2	M0	IIIA	NON	III	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
32	T4a	N3a	M0	IIIB	NON	II	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
33	T4a	N1	M0	IIIA	NON	III	ABSENT	PRESENT	YES	1	GOOD	0	0	NEGATIVE	NEGATIVE
34	T3	N0	M0	IIA	NON	II	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
35	T3	N3a	M0	IIIB	DYSPLASIA	II	PRESENT	ABSENT	NO	.	.	3	3	POSITIVE	POSITIVE
36	T4a	N2	M0	IIIA	NON	III	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
37	T4a	N3a	M0	IIIB	NON	III	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
38	T1a	N0	M0	IIA	NON	III	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
39	T4a	N3a	M0	IIIB	NON	III	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE

40	T4a	N3a	M0	IIIB	NON	II	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
41	T3	N0	M0	IIA	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
42	T3	N1	M0	IIB	DYSPLASIA	II	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
43	T3	N0	M0	IIA	NON	II	ABSENT	PRESENT	YES	1	POOR	0	0	NEGATIVE	NEGATIVE
44	T4a	N3a	M0	IIIB	NON	III	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
45	T4a	N3b	M0	IIIC	NON	II	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
46	T4a	N1	M0	IIIA	NON	III	ABSENT	ABSENT	YES	2	POOR	0	0	NEGATIVE	NEGATIVE
47	T1b	N1	M0	IB	IM	II	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
48	T4a	N1	M0	IIIA	NON	III	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
49	T4a	N3b	M0	IIIC	NON	III	PRESENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
50	T2	N2	M0	IIB	IM	II	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
51	T4a	N2	M0	IIIA	NON	III	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
52	T3	N2	M0	IIIA	NON	II	ABSENT	PRESENT	YES	3	POOR	1	0	NEGATIVE	NEGATIVE
53	T3	N1	M0	IIB	DYSPLASIA	II	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
54	T1a	N1	M0	IIB	NON	III	ABSENT	ABSENT	YES	1	GOOD	0	0	NEGATIVE	NEGATIVE
55	T2	N0	M0	IB	NON	II	ABSENT	ABSENT	YES	1	GOOD	0	0	NEGATIVE	NEGATIVE
56	T1b	N0	M0	IA	NON	III	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
57	T3	N3b	M0	IIIC	H.PYLORI	III	PRESENT	ABSENT	YES	3	POOR	0	0	NEGATIVE	NEGATIVE
58	T3	N1	M0	IIB	NON	III	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
59	T3	N3a	M0	IIIB	NON	II	PRESENT	ABSENT	NO	.	.	3	3	POSITIVE	POSITIVE
60	T3	N3a	M0	IIIB	NON	III	PRESENT	PRESENT	NO	.	.	3	3	POSITIVE	POSITIVE
61	T2	N0	M0	IB	H.PYLORI	II	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
62	T4a	N1	M0	IIIA	NON	III	PRESENT	PRESENT	YES	2	POOR	3	0	POSITIVE	NEGATIVE
63	T3	N2	M0	IIIA	H.PYLORI	II	ABSENT	PRESENT	YES	3	POOR	0	0	NEGATIVE	NEGATIVE
64	T3	N2	M0	IIIA	NON	III	ABSENT	ABSENT	YES	3	POOR	3	0	POSITIVE	NEGATIVE
65	T4a	N2	M0	IIIA	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
66	T2	N0	M0	IB	IM	II	ABSENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
67	T3	N0	M0	IIA	NON	II	PRESENT	ABSENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
68	T2	N2	M0	IIB	NON	II	PRESENT	ABSENT	YES	1	GOOD	0	3	NEGATIVE	POSITIVE
69	T4a	N2	M0	IIIA	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
70	T4a	N2	M0	IIIA	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE
71	T3	N3a	M0	IIIA	H.PYLORI	III	ABSENT	PRESENT	YES	3	POOR	0	0	NEGATIVE	NEGATIVE
72	T4a	N2	M0	IIIA	NON	III	ABSENT	PRESENT	NO	.	.	0	0	NEGATIVE	NEGATIVE